HUMAN TOXOPLASMOSIS

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Proceedings of the Conference on
Clinical Aspects and Diagnostic Problems of Toxoplasmosis in Paediatrics
at The VIII International Congress of Paediatrics
Copenhagen, 1956
Revised and edited 1959

Edited by
J. CHR. SIIM, M.D.
Director, The Toxoplasmosis Department
State Serum Institute, Copenhagen
Denmark

MUNKSGAARD . COPENHAGEN

INTRODUCTION

During the VIII International Congress of Paediatrics in Copenhagen in July 1956, a special conference was devoted to *The Clinical Aspects and Diagnostic Problems of Toxoplasmosis in Paediatrics*. The aim of the meeting was to bring together specialists engaged in studies on toxoplasmosis, in order to make known the clear-cut facts concerning the present knowledge of acquired and congenital toxoplasmosis.

Representatives from twelve countries attended this conference, which was organized by Dr. J. Chr. Siim.

The classical form of congenital toxoplasmosis, as well as the disease with minimal symptoms and signs, were discussed on the first day Then followed an account of the form which occurs most commonly, acquired toxoplasmosis with lymphadenopathy, special mention being made of the clinical symptomatology and the procedures used for its routine laboratory diagnosis. Eye toxoplasmosis, epidemiology, treatment, and laboratory diagnosis were the subjects of the papers read on the second day of the meeting

In order to meet requests for the proceedings of the conference to be published, the present publication includes the papers presented, revised and brought up-to-date.

The book is published with a view to stimulating interest in a disease which, in most countries, is not recognized to its fullest extent, either in paediatrics or in clinical medicine It will also make available, in assembled form, the references to papers published in various countries.

The Conference was made possible by valuable financial support from The King Christian X's Foundation, The Carlsberg Breweries, The Tuborg Breweries and The State Serum Institute The proceedings were published with the financial help of the Council for International Organizations of Medical Sciences (C.I.O.M S).

University Clinic of Paediatrics,
Rigshospitalet, Copenhagen

P. Plum.

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Dr. *K. Aagaard*
The State Serum Institute, Copenhagen, Danmark

Professor *F. Bamatter*
Clinique Univ de Pédiatrie Hôpital Cantonal, Geneva, Switzerland

Professor *C P. Beattie*
Dept of Bacteriology, Univ of Sheffield, Great Britain

Dr. *J. K. A. Beverley*
Dept of Bacteriology, Univ of Sheffield, Great Britain

Dr. *Rita Cardoso*
Oswaldo Cruz Institute and Univ of Brazil

Dr. *G. Desmonts*
Clinique de Puériculture, Hôpital St. Vincent de Paul, Paris, France

Dr. *D. E. Eyles*
National Institutes of Health, Laboratory of Tropical Diseases, Memphis, Tenn , USA

Professor *H A Feldman*
Dept of Preventive Medicine, State Univ of New York, Upstate Medical Center, Syracuse, N Y , USA

Professor *H. Franke*
Medical Policlinic, Univ of Wurtzburg, Germany

Dr. *J. P. Garin*
Laboratory of Protozoology and Tropical Diseases, and Hospital for Infectious Diseases, Faculty of Medicine, Univ of Lyon, France

Dr. *P. Gronroos*
Dept. of Serology and Bacteriology, Univ of Helsingfors, Finland

Dr. *Greta Hedenstrom*
County Hospital, Östersund, Sweden

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Dr. *Greta Hedenström*
County Hospital, Östersund, Sweden

Dr. *Gunnel Huldt*
State Bacteriological Laboratory, Stockholm, Sweden

Dr. *L. Jacobs*
US Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

Professor *M. Lelong*
Clinique de Puériculture, Hôpital St Vincent de Paul, Paris, France

Professor *A. B. Sabin*
The Children's Hospital Research Foundation, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio, USA

Dr. *J. Chr. Siim*
The State Serum Institute, Copenhagen, Denmark

Dr *O. Thalhammer*
Paediatric Clinic, Univ. of Vienna, Austria

Professor *P. Tolentino*
Instituto di Clinica Pediatrica, Univ of Genoa, Italy

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Paediatric Clinic, Univ. of Vienna, Austria

Professor *P. Tolentino*
Istituto di Clinica Pediatrica, Univ of Genoa, Italy

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The Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

TOXOPLASMOSIS IN PEDIATRICS. PRESENT STATUS AND PROBLEMS

Introductory Remarks at Symposium on Toxoplasmosis, Copenhagen, 1956.

ALBERT B. SABIN

Studies of the past 17 years have defined the variety of clinical manifestations which may result from prenatal and postnatal infections with toxoplasma. These syndromes will be discussed in greater detail by others this morning. It seems to me that the time has come for a quantitative estimate of the role of toxoplasmosis as a cause of actual disease in contradistinction to the accumulated information that is currently available on the high incidence of clinically inapparent infections. Thus it would be important to know:

1. How frequently is toxoplasmosis a cause of spontaneous abortion?
2. How much does it contribute to neonatal mortality?
3. What proportion of neonatal icterus is caused by toxoplasmosis?
4. How much does it contribute to cerebral damage of congenital origin?
5. What is its quantitative role in convulsions of unknown etiology and of mental retardation in childhood?
6. What proportion of undiagnosed syndromes with lymphadenopathy is caused by toxoplasmosis?
7. What proportion of acute encephalitis for which no other etiologic agents can be demonstrated is caused by toxoplasmosis?
8. What proportion of chorioretinitis of unknown etiology in childhood is actually caused by toxoplasmosis?

As for the epidemiology of toxoplasmosis, it is still a puzzle to me how so many become infected when there is no good evidence of appreciable spread from man to man or widespread dissemination by an insect vector. Although there is no adequate evidence against the specificity of the dye test for toxoplasma antibody, one still wonders whether some other agents may have an antigen in common with toxoplasma to account for the extraordinary high incidence of this antibody that is found in various human populations all over the world. In this respect, it seems to me one cannot rely too much on the titers of antibody that are detected because proved toxoplasma infections have been found after many years occasionally to be associated with titers as low as 1:4 or even less.

III TREATMENT OF TOXOPLASMOSIS

IV OPHTHALMOLOGICAL ASPECTS OF TOXOPLASMOSIS

V EPIDEMIOLOGICAL ASPECTS OF TOXOPLASMOSIS

VI LABORATORY DIAGNOSIS OF TOXOPLASMOSIS

CONGENITAL TOXOPLASMOSIS

There are a number of other questions that naturally come to mind. Is there anything we can do in a *practical* way to prevent congenital toxoplasmosis? And what is the best thing to do in the way of therapy when a newborn child presents the syndrome of icterus, hepato-splenomegaly, and rash – a combination that may lead the clinician to suspect toxoplasmosis?

Among the problems for future research it seems to me that great attention needs to be paid to the factors that may play a part in the reactivation of latent "pseudocystic" toxoplasmosis, as well as to the chemotherapy for the eradication of pseudocysts.

Although the studies thus far have provided us more with understanding than with means of prevention or practical methods of therapy of infections in the advanced stages, it is still gratifying to be able to make a diagnosis of congenital toxoplasmosis, if for no other reason than the possibility that it gives us of telling the parents that future children will not be afflicted with the same disease.

Clinique de Puériculture, Hôpital S^t Vincent de Paul, Paris, France

CONGENITAL TOXOPLASMOSIS

Introduction

MARCEL LELONG

Entre la découverte de la toxoplasmose par Charles *Nicolle et Manceaux* en 1908 et la démonstration de la toxoplasmose humaine par *Wolf, Cowen et Paige* en 1930 il s'est écoulé près d'une trentaine d'années La toxoplasmose congénitale fut la première décrite et voici que la toxoplasmose acquise nous livre ses secrets. Comme remarque préliminaire à nos discussions, peut-être n'est-il pas déplacé de nous demander s'il est vraiment légitime de séparer fondamentalement une toxoplasmose congénitale et une toxoplasmose acquise.

De toute évidence la toxoplasmose n'est jamais héréditaire; elle est toujours acquise et la toxoplasmose dite congénitale n'est rien d'autre qu'une toxoplasmose acquise pré-natale. La toxoplasmose pré-natale ne se sépare de la toxoplasmose post-natale que

- par la date de la contamination. la période foetale;
- par le terrain· des tissus embryonnaires particulièrement sensibles;
- par la porte d'entrée: le cordon, ce qui implique une invasion hématogène donc d'emblée généralisée;
- et enfin par ce fait d'importance considérable. le début de la maladie se passant dans la clandestinité de la vie intra-utérine, échappe à nos moyens d'investigation: ce que nous n'avons pas vu n'en a pas moins existé.

L'infection acquise in utéro obéit aux mêmes lois évolutives que la maladie acquise; elle parcourt le même chemin, passant du stade des lésions diffuses parasitémiques a celui des lésions régionales ou locales, elle suit les mêmes étapes. incubation, invasion, état, déclin, terminaison par la guérison ou la mort. Et c'est ici l'occasion de méditer cette profonde pensée émise dès 1828 par Billard, un des ancêtres de la pédiatrie Francaise: quand il vient au monde le nouveau-né, déjà vieux de neuf mois, peut être. ou bien-portant, ou blessé, ou malade; s'il est malade il peut être soit encore en évolution, soit convalescent, soit guéri, s'il est guéri, il peut l'être avec ou sans séquelles.

Ces éventualités, la toxoplasmose les réalise chez le nouveau-né.

Celui-ci s'il a été infecté suffisamment tôt pendant la vie intra-utérine a eu le temps de parcourir avant la naissance toutes les phases de la maladie et dans ce cas peut venir au monde guérit. Sans séquelles n'est peut-être pas une

ans un taux non significatif d'activité (anticorps lytiques) ou même nuls (anticorps fixateurs du complément).

Dans sa durée la notion de latence congénitale soulève aussi des problèmes. D'après ce que nous savons cette durée est courte et ne dépasse guère quelques semaines à 2 ou 3 mois. Avec *Rossier et Desmonts*, chez un prématuré normal à la naissance, nous avons vus une chorio-rétinite apparaître à trois mois On peut se demander si cette latence ne peut pas dans certains cas être de durée plus longue, ce qui pose la question de l'existence d'une toxoplasmose congénitale tardive, d'une part; – et, d'autre part, celle des facteurs éventuels de réactivation (maladies intercurrentes, modificateurs de l'allergie etc....). De ces facteurs il semble bien qu'on puisse exclure la grossesse, puisque quand une gestation a donné un enfant atteint de toxoplasmose, la grossesse suivante est normale.

Les rapports de la toxoplasmose congénitale et de la toxoplasmose acquise soulèvent bien d'autres problèmes que nous ne pouvons envisager ici. Par exemple. a partir de quel âge, chez un enfant jeune, peut-on cesser de parler de toxoplasmose congénitale pour envisager la toxoplasmose acquise? Quelle est la ligne de démarcation entre les deux domaines?

Il est curieux aussi de noter que, tandis que la connaissance de la syphilis a progressé de la syphilis acquise à la syphilis congénitale, celle de la toxoplasmose est allée de la toxoplasmose congénitale à la toxoplasmose acquise. A cela il y a une raison profonde, tout se passe comme si l'être humain adulte était doué d'une forte résistance innée vis à vis de la toxoplasmose. Chez l'adulte et même de grand enfant la toxoplasmose est le plus souvent inapparente l'immunité se déclenchant en même temps que l'infection Dans certains cas, que les belles recherches de *J Chr Sim*, ont contribué à faire connaître, des signes minimes cependant peuvent être décelés.

Mais le foetus n'a pas cette heureuse résistance ainsi s'explique le contraste saisissant entre un nouveau-né durement frappé et une mère en apparence bien portante. Ainsi se dégage cette conclusion pratique d'importance capitale: tout l'intérêt de la toxoplasmose humaine est dans l'etude des moyens de dépistage systématiques de la toxoplasmose acquise de la jeune femme enceinte. En effet la connaissance de la toxoplasmose acquise, dans ses formes atténuées et minimales, comme dans sa forme inapparente, uniquement sérologiques, va rendre possible l'organisation du dépistage (clinique, ophtalmoscopique, radiologique, sérologique) de la toxoplasmose initiale de la jeune femme qui commence une grossesse. Conjugué avec les progrès de la chimiothérapie, ce dépistage pré-natal fera disparaître la toxoplasmose grave du nouveau-né et du nourrisson· la réalisation de cette espérance sera la meilleure récompense des chercheurs.

impossibilité, quoique d'après ce que nous savons cette hypothèse soit pour le moment peu vraisemblable. Avec séquelles graves, définitives, profondément destructrices de territoires étendus du cerveau, de l'oeil, de la moelle, c'est ce que nous voyons habituellement: l'incendie est éteint et le clinicien ne peut, hélas, que faire l'inventaire des dégâts.

Il peut arriver que la maladie, encore active n'ait pas au moment de naissance achevé son cycle. Le nouveau-né, s'il a franchi le stade des lésions parasitémiques et pluri-viscérales, vient au monde en présentant des lésions localisées, habituellement cérébro-oculo-médullaires. Le tableau est alors celui d'une *encéphalo-myélo-pathie subaigue, caractérisé par la tétrade* bien connue: hydrocéphalie, chorio-rétinite, calcifications intracraniennes, troubles psycho-moteurs importants. La maladie est-elle moins ancienne, on peut voir s'ajouter au syndrome précédent, ou même exister seuls des signes attestant une atteinte plus diffuse parce que plus proche de la phase parasitémique: exanthéme, pneumonie, hépatite avec ou sans ictère. Ici encore les lésions inflammatoires, nécrotiques ou scléreuses, profondément destructrices font de l'enfant s'il survit un lamentable déchet humain que restera à charge à sa famille ou à la société.

Enfin il faut considérer une troisième éventualité. Si le foetus a été infecté très peu de temps avant l'accouchement, le nouveau-né peut se présenter extérieurement comme un enfant normal, la maladie n'étant qu'a sa phase d'incubation. Dans ce cas l'infection est inaparente et c'est ultérieurement, plus ou moins longtemps après la naissance que, comme une toxoplasmose acquise post-natale, elle déroulera son cycle, sans qu'aucun de ses stades ne réalise en vérité un type clinique special à l'infection congénitale. En ce sens il existe donc une toxoplasmose latente congénitale, cette latence devant être définie dans sa qualité comme dans sa durée.

Dans sa qualité, la latence n'est que relative et dépend évidement de la pénétrance plus ou moins grande de nos moyens d'investigation. Elle peut-être seulement clinique, l'examen clinique seul étant négatif alors que l'examen ophtalmoscopique montre une chorio-rétinite et l'examen radiologique des calcifications intra-craniennes. Elle peut-être à la fois clinique, ophtalmoscopique et radiologique, seuls les tests biologiques étant positifs. Mais alors se pose le problème de l'interprétation des signes biologiques isolés chez le nouveau-né. Selon nos constatations le nouveau-né possède les anticorps de sa mère, lytiques ou fixateurs, parfois même à un taux plus fort. Jusqu'a trois mois les réactions constatées traduisent plutot les anticorps maternels transmis; ceux-ci disparaissent peu à peu et ce n'est qu'apres neuf mois que les anticorps décelés appartiennent *surement à l'enfant. En principe dans le cas* de la toxoplasmose congénitale avérée, les réactions restent positives chez l'enfant à un taux élevé pendant un ou deux ans, puis elle diminuent progressivement, le titre des anticorps décroissant pour atteindre vers trois ou cinq

absence of all traces of inflammation makes diagnosis difficult, even by histopathological examination.

The existence of malformations in such cases also makes interpretation difficult. The pathogenesis of congenital anomalies in infants with demonstrable toxoplasmic antibodies was pointed out by the first observers, and is also far from elucidated and still a question of great importance. We have observed personally a newborn child with teleencephalocelus dating from the organogenetic stage, and other grave malformations, in which the Sabin-Feldman test gave a titer of 1:16000 and the complement fixation test 1:10, whilst the mother had titers of 1 128 and 1:5 respectively. A neurohistological study of this case showed complex dysplasia without recent reactionary signs or damage to the brain and its surrounding tissue.

As regards the oligosymptomatological cases of congenital toxoplasmosis, great prudence would seem to be essential in establishing the relation of cause and effect. The older the age at which the child becomes afflicted with psychomotoric retardation or cerebral insufficiency, the more difficult it is to interpret the low antibody titers as an indication of congenital toxoplasmosis, since these titers often seem to be just as high in healthy children of the same ages.

The long survival time of toxoplasma in the organism has been confirmed by us in a child of 5 years of age suffering from a chronic congenital toxoplasmic encephalitis. By inoculating white mice we were able to isolate toxoplasma from small cortical foci. In any case, the antibody titers in the child and the mother would not have indicated the nature of the disease.

Our attention has been focussed, clinically, on the neurological signs of congenital toxoplasmosis, particularly the motor phenomena, which are rather peculiar in some patients, and a film has been made of our observations.

Neither the motor involvement nor the electro-encephalographic anomalies can be considered as specific, but realization of them is of value each time they are encountered in the incomplete forms of the disease, when assembling the accessory elements for support of the diagnosis.

Evaluation of the clinical and serological results is the basis for the diagnosis of congenital toxoplasmosis In doubtful cases, it is also necessary to try to demonstrate the infecting agent.

Clinique Universitaire de Pédiatrie, Hôpital Cantonal,
Geneva, Switzerland

CONGENITAL TOXOPLASMOSIS

F. BAMATTER

SUMMARY[1]

In 1952 it appeared that the congenital form of toxoplasmosis occurred more frequently than the acquired form. To-day the reverse is the case, due mainly to the description by *Siim* of the glandular form of the disease.

The clinical symptoms of congenital toxoplasmosis are, as mentioned, already well known, and it has been established that the neuro-ocular syndrome is of much greater importance than the visceral phenomena.

Classification of the frequency of the most important manifestations of the disease among 271 cases examined shows the following distribution: ocular symptoms 89.7 %, neurological symptoms 66.8 %, intracerebral calcification 56.8 %, hydrocephalus 36 5 %. The frequency distribution of the different ocular symptoms in 243 cases was: bilateral choriodıtis 65.7 %, unilateral choriodıtis 34 3 %, microphthalmia 22.6 %, nystagmus 22.6 %, *cataract 8.2 %, iritis and posterior synechia 7.7 %, pupillary membranes* 4.5 %, vitrous changes 11.1 %.

A clinical and pathological symptomatology similar to that of congenital toxoplasmosis is found in the Sabin-Feldman syndrome, in which the serological antibodies are missing. Our experience has shown that it is possible that a relatively low dye test titer, e. g. 1.64, may indicate the presence of congenital toxoplasmosis in newborn infants.

Prenatal toxoplasmic meningo-encephalitis shows a tendency to develop haemorrhagic necrosis (xanthochromia) and hydrocephalus resulting in the almost complete destruction of the cerebral tissue (burnt-out cases). In addition to these extreme cases, we have also seen all the intermediary forms, right down to almost no affection of the brain (from the functional point of view also). Toxoplasmic encephalitis of long duration can be detected only by X-ray because of the presence of one or two small areas of intracerebral calcification.

The pathological consequences of meningo-encephalomyelitis are often very slight after several months, and as a rule after several years; and the

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absence of all traces of inflammation makes diagnosis difficult, even by histopathological examination.

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the parasite from the rabbit to pigeon, and, in 1911, when he discovered the spontaneous infection of the pigeon and dog, he identified the parasite with that of the rabbit. In 1914, *Arantes* transferred parasites from dog to pigeon. *Mesnil* (1918) published experiences of cross-immunity and, in 1939, *Sabin* showed the identity of *Toxoplasma* from man and from other animals. In 1940, *Wolfson*, who also had transmitted *Toxoplasma* from mammals to birds, claimed to have proof of the spontaneous transmission of the parasite from birds to mammals; this proof has been given, at the same time and independently, by one of us (*Nery-Guimarães*, 1942) and by *Nobrega & Reis* (the strain from pigeon has been transmitted to dogs, cats, rabbits, guinea pigs and mice) Today, all authors agree that the so-called "species" of *Toxoplasma* are synonymous with *Toxoplasma gondii, Nicolle & Manceaux*, 1909.

(C) *Biological characteristics of Toxoplasma*

The most important characteristics of the biology of this protozoa "sui generis" are its universal geographical distribution, its capacity for infecting animals from different zoological classes (lack of specificity) and its capacity for parasitism in practically all the animal tissues Of practical value to the definite diagnosis of *Toxoplasma* is its pathogenic action on several different hosts, because other parasites capable of interfering in the diagnosis (*Encephalitozoon, Sarcocystis, Schizotrypanum cruzi, Leishmania*, etc) are more specific. Such parasites must be considered, especially in the attempts to isolate the *Toxoplasma* from man and in the studies of experimental toxoplasmosis.

Another peculiar aspect of the biology of *Toxoplasma* is its necessary intracellular parasitism (*Sabin & Olitsky*, 1937) All the attemps to cultivate it on the synthetic artificial culture media suitable for protozoa and fungi have up to now been unsuccessful

In 1929, *Levaditi et al.* cultivated *Toxoplasma* in chicken embryo and in tissue culture In 1937, *Sabin & Olitsky* and in 1942, *Nery-Guimarães & Meyer* also managed to cultivate it in tissue culture; the latter have been able to keep the parasite in such cultures for several months, without loss of virulence, and confirmed the incapacity of *Toxoplasma* for reproduction outside the cell. In addition they observed the mobility of *Toxoplasma*, until then denied by many authors, as well as its active penetration into the cells, where, by successive bipartitions, they organize themselves as colonies (pseudocysts). In that paper an "organella" in the *Toxoplasma* (trombicula) has been described for the first time; this "trombicula" is probably connected with the flagella-like filopodium ("geisselartiges Filopodium") described by *Westphal* (1954) and it is surely related to the "conoid" of *Gustafson et al.* (1954), seen by these latter authors at the electron microscope; its presence

Oswaldo Cruz Institute and University of Brazil,
Oswaldo Cruz Institute, and Fernandes Figueira Institute,
Geneva, Switzerland

CONGENITAL TOXOPLASMOSIS

RITA ALVES DE ALMEIDA CARDOSO, FELIPE NERY-GUIMARÃES,
AND APARECIDA PINTO GARCIA

Toxoplasmosis has become very important in the last fifteen years, extending from the field of the pathological anatomy to representing now a daily subject of consideration for pediatricians, ophthalmologists, neurologists, gynecologists and general physicians.

Since 1950, on the basis of serological surveys. this importance has been overestimated to the extent of presenting toxoplasmosis as one of the most common human infections. However, it is really a serious medical-social problem, demanding wide research to clear up the multiple aspects which are still obscure.

Before reporting our observations based on 6 cases of congenital toxoplasmosis, we could like to give a historical review of toxoplasmosis, recalling some pioneer contributions from Brazilian authors to the study of such an interesting chapter of medicine.

(A) *Discovery of the parasite*

Toxoplasma organisms were reported simultaneously by *Nicolle & Manceaux* (1908), in Tunis (in the gondi, a north African rodent – *Ctenodactylus gondi, Pallas*, 1778) and by *Splendore* (1908), in São Paulo (in the rabbit). It must be remembered, however, that although *Splendore*, already in his first publication, classified the parasite as "a new parasitic protozoa of the rabbit", the French authors first related the parasite to "the Leishman corpuscules or closed organisms" and a year later qualified it as a "new protozoa of the gondi", calling it *Toxoplasma gondii.*

(B) *Identity of the so-called species of Toxoplasma*

For a long time the system of classifying species of *Toxoplasma* on the basis of its host prevailed. However, several authors, in Brazil and in France, have always considered *Toxoplasma* to be a single species (*Carini*, 1911; *Arantes*, 1914; *Chatton & Blanc*, 1917; *Mesnil*, 1918, etc.). In 1909, Carini transferred

were negative. At the 6th month, she presented "intense fever during 30 days", received on that occasion 4,800,000 I.U. of penicillin, after which the temperature became normal; after some days, she stopped feeling the fetal movements and, after 5 days, the patient showed a bloody vaginal discharge. At that time, examination revealed a uterus corresponding to a pregnancy of 6–7 months and a living fetus, with 140 heart beats per minute. The clinical diagnosis was "premature detachment of placenta". The duration of the delivery was 9 hours; the fetus was stillborn. Normal puerperium, the patient being discharged in apparently perfect health It is important to observe that the patient reported that a few days before her fever, a dead dog, in an advanced state of putrefaction, was discovered close to the house in which she lived. The patient had no direct contact with the dead dog, which was buried in the district where she took walks. She affirmed that she had no contact with other animals.

From the necropsy of the fetus resulted the following *anatomical diagnosis:* Congenital toxoplasmosis *Encephalitis necrotisans.* Diffuse myocarditis. Focal peri- and endocarditis. Interstitial nephritis and perinephritis Chorioretinitis. Placentitis. Omphalitis. Necrotic foci in the adrenal glands. Erythropoiesis in liver, spleen, kidneys, adrenal glands and placenta Presence of *Toxoplasma* in brain, eye, heart, kidney, placenta and umbilical cord.

Case 2 (A 449 – "Carmela Dutra" Lying-in Hospital)

Maternal history. F.A B., 23 years old, one previous pregnancy, normal term birth, child normal up to now. Serological test for syphilis, negative. The 2nd pregnancy was normal until the 6th month, when the patient showed fever during 3 days; after the fever, she began to lose a small quantity of blood through the vulva After 30 days profuse hemorrhage made necessary a Cesarian section because there was suspicion of "placenta previa". The liveborn baby died 15 minutes after the birth. The necropsy of the baby gave the following *anatomical diagnosis* Congenital toxoplasmosis. Prematurity. *Encephalitis necroticans* with calcification Hypernephritis. Interstitial focal myocarditis Interstitial pneumonia and undeveloped lungs. Focal placentitis and omphalitis. Pronounced erythropoiesis in liver and in spleen Hepato- and splenomegaly. Presence of "pseudocysts" of *Toxoplasma* in the adrenal glands.

Case 3 (A 154 – "Carmela Dutra" Lying-in Hospital).

Maternal history. C.S.A., 26 years old. Serological tests for syphilis, negative.

Before this pregnancy (her first) the patient presented high temperature, with headache and coryza, for 2 days (sic). The pregnancy was normal, the delivery was spontaneous and at term, but the fetus was stillborn, macerated

could mean not only motor functional activities, but possibly also activities of trophic sort.

(D) *Discovery of human toxoplasmosis*

Human toxoplasmosis was first reported in Prague, in 1923, by *Janku*, who found parasites in a child's eye, relating them to *Sporozoa*. That child presented also encephalitis, but the nervous system was not subjected to microscopical examination.

Four years later (1927), in Rio de Janeiro, Brazil, *Magarinos Torres* described "a new human disease" and its anatomical picture, emphasizing the meningoencephalitis and the myocarditis, and establishing for the first time the congenital character of the infection; the same author related the parasites found in the nervous system, in the myocardium, in the skeletal muscles and in the derm, to the *Toxoplasma* and the *Encephalitozoon*. However, *Wolf & Cowen* (1937) identified such parasites as *Toxoplasma*, by making a review on the cases reported in the literature.

Material and Technique

Our material consists of six cases of toxoplasmosis observed in stillborn and newborn babies at three different hospitals in Rio de Janeiro, Brazil; they were found in a total of 1200 necropsies, an incidence of 0.5 %.

Among the six cases, 4 were premature babies liveborn at 6th–8th gestational month and 2 were stillborn (1 premature and 1 at term). In all those cases, the diagnosis was based on the detection of the parasite in tissues and, in Case 1, *Toxoplasma* were isolated from the necrotic material found in the cranial cavity. This strain of *Toxoplasma*, which is pathogenic to pigeon and guinea-pig, is preserved by successive transfers in mice and has been used in the dye test.

We used several methods for the histological sections: hematoxylin-eosin, Giemsa, periodic-acid-Schiff, von Kossa and, in the material of Case 4, in which the maternal serological tests for syphilis were positive, we impregnated it by the Levaditi method, in search of Treponema.

Summary of the data obtained from the cases:

Case 1 (N.F. 167 – "General Vargas" Hospital).

Maternal history: M.F.M., 23 years old, in good state of health, three previous pregnancies resulting in normal term babies, the children normal up to now. The 4th pregnancy, under medical control, was normal until the 6th month. Serological tests for syphilis (Wassermann-Eagle, Kahn, Kline)

were negative. At the 6th month, she presented "intense fever during 30 days", received on that occasion 4,800,000 I.U. of penicillin, after which the temperature became normal, after some days, she stopped feeling the fetal movements and, after 5 days, the patient showed a bloody vaginal discharge. At that time, examination revealed a uterus corresponding to a pregnancy of 6–7 months and a living fetus, with 140 heart beats per minute. The clinical diagnosis was "premature detachment of placenta". The duration of the delivery was 9 hours, the fetus was stillborn Normal puerperium, the patient being discharged in apparently perfect health. It is important to observe that the patient reported that a few days before her fever, a dead dog, in an advanced state of putrefaction, was discovered close to the house in which she lived. The patient had no direct contact with the dead dog, which was buried in the district where she took walks. She affirmed that she had no contact with other animals.

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Before this pregnancy (her first) the patient presented high temperature, with headache and coryza, for 2 days (sic). The pregnancy was normal, the delivery was spontaneous and at term, but the fetus was stillborn, macerated

and hydropic. The necropsy resulted in the following *Anatomical diagnosis:* Congenital toxoplasmosis. Pronounced maceration. *Encephalitis necroticans.* Enlargement of spleen, liver and kidneys. Placentitis and relative immaturity of placenta. Interstitial myocarditis and nephritis.

Presence of "pseudocysts" of *Toxoplasma* in the adrenal glands.

Case 4 (A. 214 – Instituto de Puericultura).

Maternal history: B.L.F., 28 years old, one previous pregnancy, normal child. Serological tests for syphilis (Wassermann and Kahn) positive, at the 8th month of the 2nd pregnancy, on that occasion, the patient presented hemorrhage through the vulva. Abnormal delivery caused by "placenta previa". The fetus was born with cyanosis and started to breath only after a period of resuscitation; the cyanosis continued and was followed by fever (40° C) and disseminated rales in both lungs. The baby lived 18 hours.

The necropsy gave the following *anatomical diagnosis*· Congenital toxoplasmosis. Prematurity. *Encephalitis necroticans*, with calcification. Interstitial focal myocarditis and endocarditis. Diffuse pericarditis. Pronounced erythropoiesis and congestion in liver and spleen Hepato-and splenomegaly. Focal erythropoiesis in kidneys, in adrenal glands and in lungs

Presence of many "pseudocysts" of *Toxoplasma* in the brain.

Case 5 (N F. 63 – "General Vargas" Hospital).

Maternal history: not well verified. Premature delivery at the 7th month of pregnancy The baby died when she was 31 days old. The necropsy gave the following *anatomical diagnosis*· Congenital toxoplasmosis. Prematurity. Focal *encephalitis necroticans* (around the ventricles). Cerebrospinal purulent leptomeningitis. Atrophy of the thymus. Presence of "pseudocysts" and isolated forms of *Toxoplasma* in the brain

Case 6 (N.F. 88 – "General Vargas" Hospital).

Maternal history· – Not well verified Premature delivery at the 6th month of pregnancy. The baby died when she was 14 days old, with signs of respiratory disease.

Anatomical diagnosis. Toxoplasmic encephalitis. Pneumonia Partial atelectasis of lungs. Atrophy of thymus. Prematurity.

DISCUSSION

In the six cases of congenital toxoplasmosis that we had the opportunity to verify, some facts are of interest not only strictly anatomically but also have certain value for the better understanding of the disease.

First, we want to call attention to the presence of a sudden high fever

during or a little before pregnancy, in the 4 cases in which the maternal history was thoroughly studied. This fever, which was preceded by a normal beginning of pregnancy, had relatively rapid remission, but in 2 cases was immediately followed by genital hemorrhage and premature delivery, although the puerperium had been apparently normal. It is possible that such history could be indicative of the beginning of infection in the maternal organism, which is the reason why we draw attention to the need of careful anamnesis, specially in the cases diagnosed as inapparent infection.

Another fact to notice was that, in 5 of our cases, the event of premature delivery happened always between the 6th and the 8th month of pregnancy, and the only term fetus was delivered in an advanced stage of maceration.

The above mentioned facts could agree with the opinion of *Frenkel* (1949), when he declared that "primary infection of the pregnant mother appears more likely to be the commoner mode of fetal toxoplasmic infection", and those facts would disagree with *Weinman* (1952) who believes that the transmission of *Toxoplasma* to the fetus is more frequent through a pregnant woman with chronic disease, and who says "that infection contracted during pregnancy may and probably does happen from time to time . ."

Still in connection with the transmission of toxoplasmosis, we want to note the verification of inflammatory lesions in the placental villi and in the umbilical cord in 3 of the 4 cases in which such organs were examined microscopically. In Case 1, we found several pseudocysts of *Toxoplasma* in the placenta and the fibroblasts of Wharton's jelly were particularly rich in isolated forms and in colonies of *Toxoplasma*; the easy multiplication of the parasite in that tissue attracts attention and even suggests its utilization for cultivation of *Toxoplasma.*

The confirmation of *Toxoplasma* in human placenta was made only recently by *Christen et al.* (1951) and by *Neghme* (1952); it is not frequent in the literature, which gives some value to our present verification

It is interesting to accentuate that in 4 of our cases, we observed inflammatory lesions, proved many times to be specific, in the thoraxic and abdominal organs (heart, lungs, kidneys, adrenal glands), but the liver never presented any inflammatory lesion, although it appeared enlarged, that enlargement was due to congestion and accentuated erythropoiesis. This fact is strange, since the liver is the organ most intimately related to the umbilical cord and the placenta, through the blood.

Another observation was that provided by Case 6. There we found no gross changes that suggested toxoplasmosis, except the presence of some small necrotic foci in the cerebral nervous substance around the ventricles. There was not really enlargement of spleen or liver, and neither leptomeningitis nor hydrocephalus Such foci were first attributed to possible anoxia and are extremely similar to the anoxial softenings, even when they are ex-

amined at the microscope. Its structure is composed of a central necrotic zone, surrounded by proliferated neuroglia and of a variable deposit of calcium salts, and simulates closely the anoxial softening, when the microscopical examination is based on the common histological preparations (hematoxylin-eosin, etc.). But when we examine preparations by the Giemsa or by the P.A.S. methods, we note the presence of *Toxoplasma*, with its typical aspect or slightly changed by degeneration.

When we describe this observation, we wish to evidence the need of the search for *Toxoplasma* and related organisms, in cases of supposed pure anoxial softenings of nervous substance in children.

CONCLUSIONS

1 – *Incidence of toxoplasmosis in fetuses and newborn babies in Rio de Janeiro, Brazil.*
Among 1200 necropsies of fetuses and newborn babies we found 6 cases (0.5 %) of congenital toxoplasmosis, responsible for death in 5 cases.

2 – *Inflammatory lesions and presence of* TOXOPLASMA *in the umbilical cord and placenta.*
Inflammatory changes in umbilical cord and placenta were found in 3 patients, associated with the presence of *Toxoplasma* in one of them. This is a significant finding related to the congenital transmission of toxoplasmosis.

3 – *Macro – and microscopical similarity of toxoplasmic lesions of the nervous substance with anoxial softenings.*
In one of our cases, the cerebral lesions around the ventricles presented extreme resemblance to small softenings, and only the microscopical study by special methods revealing the presence of the parasite allowed the correct diagnosis of toxoplasmosis. It is likely that similar cases would be detected if the search for *Toxoplasma* by appropriate methods was attempted in necropsies of fetuses and newborn babies with supposed anoxial softenings.

4 – *Sudden high fever during pregnancy possibly indicative of the beginning of maternal infection.*
High temperature, possibly related to the maternal toxoplasmosis was noticed in 4 patients, during or just before the pregnancy; in 2 cases, it was soon followed by uterine bleeding and premature delivery.

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Instituto di Clinica Pediatrica, University of Genoa, Italy

CONGENITAL TOXOPLASMOSIS

P. Tolentino

The first cases of congenital toxoplasmosis in Italy were described by me and the ophthalmologist *Bucalossi* in a meeting of the Italian Society of Pediatrics, Ligurian section, in 1947. The diagnosis was serologically verified in the following year, when I came into possession of the Bk strain of toxoplasma (isolated by Winsser) which allowed me to begin systematic serological researches in suspicious cases. First I used the old rabbit neutralization test, and then, since 1949, the dye test, which I was able to carry out correctly at once, thanks to direct personal instruction by Dr. *Sabin*, and to having found a good donor of the accessory factor. Thus, over a thousand sera were examined by me in the following years, from patients of the Pediatric Clinic of Genoa and of other Clinics all over Italy I was thus able to prove the existence of several cases of congenital toxoplasmosis. I shall, however, illustrate here only the cases I was able to observe directly even from a clinical point of view, and I shall demonstrate their radiologic, ophthalmologic, and electroencephalographic pictures.

I think that a unitary classification, according to *Frenkel's* theory, may be suitable both for congenital and for acquired toxoplasmosis, but I shall follow here a more schematic distinction into active forms, showing signs of the evolutive disease, and inactive ones, that is either symptomatic (convulsions, blindness) or asymptomatic sequels (cerebral calcifications occasionally discovered or unsuspected chorioretinitis in healthy children). We may also distinguish cases with complete symptomatology (chorioretinitis, calcifications, hydrocephaly and convulsions) and incomplete ones

Among the active forms, I remember one of our first cases, a female infant who at 6 months of age was no longer able to support her head or to sit up, ceased to smile and take interest in her surroundings and began to show convulsive seizures. She looked hydrocephalic and oligophrenic; she had a typical bilateral chorioretinitis with some spots in the atrophic phase and others in the active stage, with evident development and extension of them during the following months There were no calcifications, and the case is therefore classified as incomplete; the neutralization test was strongly positive in the child and the mother.

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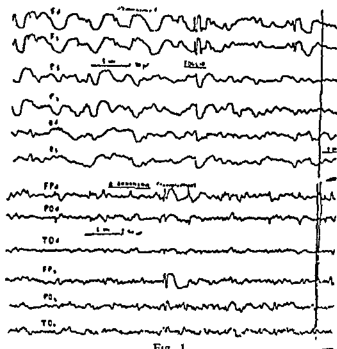


Fig 1

The following is an example of an inactive case: a girl I saw for the first time at 8 years of age, suffered from the first years of her life from left hemiparesis, with hypertony, dysmetry, astereognosy and absence of tactile discrimination on the same side, preceded by *sensitive aura, and without loss of* consciousness; there were spots of atrophic chorioretinitis in the left eye, and numerous endocranial calcifications; one of them was in the area of the cerebellum, others in the right parietal region, the dye test was positive with a low titer (1:16), as might be expected.

This was an example of clinically apparent sequels. The following are examples of inapparent or almost inapparent ones.

A 12-year-old girl, who could never see well, is able to attend school, has never had convulsions, general and neurological examinations are both negative; examination of the fundus oculi demonstrates, however, an atrophic chorioretinitis in the macular region of both eyes, of pseudo-colobomatous type, with much pigment arranged in irregular masses; the vision, corrected by lenses, is 4/10, but there is a central scotoma of 12×7 degrees; the X-ray picture of the skull demonstrates numerous calcifications spread over the whole encephalic mass; the dye test is positive with low titer.

A 10-year-old healthy boy, with a few signs of hypogenitalism, is occasionally submitted to X-ray of the skull in order to examine the sellar region; the picture shows numerous calcifications of various sizes irregularly distributed in the brain; examination of the fundus oculi demonstrated spots of chorioret-

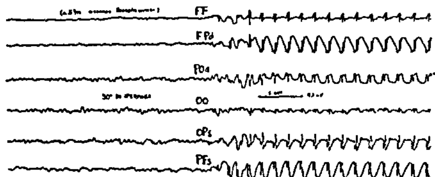


Fig 2

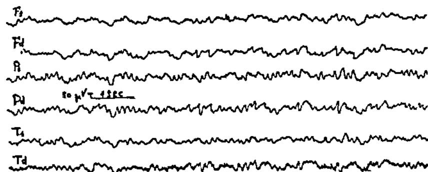


Fig 3

initis in the atrophic stage in both eyes, the dye test was 1 64, and 1 2048 in the mother (the case was published by *Di Sieno*).

The existence of incomplete cases appears documented in my own case records by some of the formal cases, as in the following: a 3-months-old male infant shows seizure type flexion-spasms, diminution of vision and hearing; his fundus oculi appears to be extensively depigmented but without any spot of chorioretinitis, there are, however, many cerebral calcifications and a slight hydrocephaly, the patient improves after treatment with sulfadiazine; the dye test was 1·512 on patient's serum and 1 2048 in his mother

Electroencephalography has greatly improved the possibility of examination of these patients, allowing us to detect even small foci of encephalitic lesions which are clinically and radiologically inevident The first cases of toxoplasmosis I studied with *Lombroso* in 1950 led us to believe that electroencephalographic lesions of toxoplasmosis may be characteristic, but further observations demonstrated that our opinion was wrong. The modifications in EEG. pattern are, in fact, of various types· we can see a dysrythmic pattern type "petit mal variant" in frontal derivations (Fig. 1) as in our first

cases; or a typical dysrythmic discharge with paroxismal hypersynchronism of spikes and waves 3 c/s, suggesting the existence of periventricular lesions (Fig. 2) as in another case, an 8-year-old girl, who had been suffering for the last 3 years from absences type "petit mal", resisting any treatment, and from chorioretinitis; or a pattern of localized focus in the parietal region, with diphasic spikes of mean voltage (Fig. 3) as in another unpublished case, showing convulsive seizures, chorioretinitis and slight cerebral calcifications.

In none of the cases observed directly by me was toxoplasma isolated or pathologically demonstrated. However, this was so in some other cases of toxoplasmosis observed by other Italian authors.

The case published by *Mulè* concerned a 17-day-old baby, affected by convulsions, microphthalmia, purulent discharge of the conjunctivae, anisocoria and pupillar rigidity. Toxoplasma was isolated from the cerebrospinal fluid by using embryonated eggs; the child died and the details of the postmortem were not reported.

Ruosi's case was a 23-year-old woman, who had suffered from numerous convulsive seizures since the age of 2 years, had paresis of the left arm and showed progressive psychic deterioration; there were neither calcifications nor chorioretinitis; the cerebrospinal fluid was normal, but a toxoplasma strain was obtained by mouse inoculation.

Another case was recently described by *Baldasseroni & Francalancia*, of the Pediatric Clinic of Florence: a 2-months-old male infant, dystrophic, hypothermic, showing meningeal signs and convulsive seizures, with hydrocephaly and xantochromic cerebrospinal fluid, died 3 months later; there was a focus of chorioretinitis and diffuse calcifications of the brain, which were confirmed by the necroscopy, showing also bright zones of encephalomalacia; toxoplasmata were found in histologic preparations.

The existence of diseases simulating even the complete symptomatology of congenital toxoplasmosis and chiefly monosymptomatic forms is well known, the negative nature of serologic examinations allows us to distinguish these cases from authentic toxoplasmosis, and to carry out our research in other directions. I shall give here some examples from our case records.

A 15-day-old female baby showed cyanosis, dyspnoea, and various anomalies, *including microphthalmia, coloboma of the iris, cerebral calcifications,* convulsions, and high fever. The dye test was negative; a postmortem demonstrated diffuse inflammatory lesions of the brain, liver and lungs, with nuclear inclusions, of probable viral origin (the case has been published by *Cavalieri & Saccomani*).

Another case concerned a 7-year-old girl, with hemiparesis and hemiathetosis of the left side, and cerebral calcifications; the negative nature of the dye test allowed me to exclude the diagnosis of toxoplasmosis, and the final

diagnosis was that of calcified tuberculoma of the brain (the girl had suffered from tuberculosis miliaris at the age of 5 years).

In other cases there were no inflammatory lesions of the eye and brain but degenerative anomalies depending or associated with status dysraphicus: the chorioretinopaty of these malformations may simulate a toxoplasmic chorioretinitis. This was the case in a 3-months-old female infant affected by vomiting and convulsions, hypotony and hyperreflexia, microphtalmia at the right side and bright yellow spots of the fundus, defined by pigmented zones; the encephalography demonstrated a typical picture of agenesis of the corpus callosum. Another boy of 9 years, suffering from occasional convulsions, and showing some signs of endocraniosis, had a big spot simulating chorioretinitis, but also cerebral anomalies such as cyst of the septum pellucidum and of the cavum Vergae. In both cases the dye test was negative.

SUMMARY

The author relates cases of congenital toxoplasmosis observed by him and others in Italy, distinguishing active (evolutive) forms from inactive ones (symptomatic or asymptomatic sequels), and also cases with complete or incomplete symptomatology. Ocular findings, roentgenologic aspects of the skull and electroencephalographic pattern are summarized. Emphasis is placed on the differential diagnosis between toxoplasmosis and other infectious (viral, tbc) or non-infectious conditions (degenerative anomalies) which may simulate a toxoplasmic disease

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County Hospital, Östersund, Sweden

THE VARIABILITY OF THE COURSE OF CONGENITAL TOXOPLASMOSIS, COMMENTS ON SOME RELATIVELY MILD CASES

GRETA HEDENSTROM

Toxoplasmosis acquired after birth can be malignant or mild. There are cases with extensive involvement of the brain, the eyes, the heart and the lungs. On the other hand, there are clinically silent infections, representing the *majority of serologically recognizable cases. Mild* cases of lympadenopathy with or without fever are not unusual. Many different degrees of constitutional involvement are obviously possible where acquired toxoplasmosis is concerned.

The general concept of congenital toxoplasmosis seems, on the other hand, *to be entirely dominated by the classical severe variety of the disease. Most* cases reported suffer from a destructive meningoencephalitis, leading to cerebral calcifications, hydrocephalus and mental retardation. Very often this is combined with chorioretinitis or other severe damage of the eye. It has been suggested that the embryo might be more susceptible to toxoplasmic *infection than the human organism after birth.*

Several cases have, however, been reported where the destructive powers of the Toxoplasma organisms have been restricted to one of the patient's eyes, causing a chorioretinopathy, often accompanied by a squint In this connection, the probability of the occurrence of mild cases of congenital toxoplasmosis has been stressed by more than one author (10, 13, 18). It seems probable that cases with the classical combination of severe symptoms are more often suspected to be due to toxoplasmosis than more benign cases The fact that only few mild cases have been found may be due to their not having been looked for.

During the course of less than one year, three infants from the County of Jämtland were examined in the Pediatric Department of the County Hospital in Östersund, and found to be suffering from congenital toxoplasmosis. Two were of the classical severe variety of the disease. One girl, however, (Case 1) had as her only complaint a benign convulsive disorder. Otherwise she is healthy and well developed.

Three other children from the same part of Sweden were found to suffer from different degrees of cerebral and ophthalmic destruction. In Case 2 the child was cared for in the University Hospital in Uppsala I owe my knowledge of this case to the courtesy of Professor B. Vahlquist. In all three cases the diagnosis of congenital toxoplasmosis was probable, although it cannot be looked upon as *definitely proved.*

Case 1.

E.K.M., a girl born at term, seemed completely healthy when examined a few days after birth. Birth weight 4100 g.

She was the first-born child. Her mother had had her tonsils removed in May 1953 because of insistent infections. Three months later there was an abortion but after another 3 months she was pregnant again and gave birth to our patient in June 1954. The child was entirely breast-fed during the first 5 months of life.

Between the age of 2½ and 5 months she had six attacks of generalized convulsions. In connection with the convulsions she was unconscious for about 1 minute each time.

Since the age of 5 months she has never had any convulsions, but she has suffered from occasional minor attacks of seizures – petit mal. If acutely infected or exposed to unaccustomed surroundings, she can have several attacks a day. When there is nothing extraordinary to disturb her, she has been completely free from attacks for many weeks She has never had any anti-convulsive therapy, except in connection with the convulsions during the first 5 months of her life. Her physical and mental development has been satisfactory, in fact rapid. She could walk at the age of 11 months and speak a few words at 15 months. She is active and merry, but not restless.

She has never showed any signs of spasticity or rigidity There are no cerebral calcifications to be seen on the *X*-ray films. Her eyes are completely free from pathological signs. In her *ECG* there is nothing abnormal, nor in the one *EEG* that has as yet been performed.

A lumbal puncture at the age of 5 months gave a fluid of normal composition. All her blood values have been within normal limits

Serological data·

Child's date of birth: June 8th, 1954.

Date	Mother		Child	
	Dye test	C F T	Dye test	C F T
1954				
11/11	1/1250	1/30		(impossible to interpret)
26/11			1/250	
1955				
11/1	1/250	1/7,5	1/250	1/7,5
15/3	1/250	neg	1/1250	neg
30/6	1/250	neg	1/250	neg.
21/9	1/50	neg	1/250	neg.
8/11	1/50	neg	1/50	neg
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Case 3.

B.O.A., a boy born at term, was brought to the hospital in Östersund at the age of 2 years 3 months because of convulsions in the left part of his body, followed by several hours of unconsciousness. He had had three attacks of convulsions previously at the age of 5 months, 1 year, and 1 year 5 months respectively.

There were no definite signs of mental retardation The boy could walk at the age of 1 year 6 months and could speak a few words at 1 year 10 months.

A lumbal puncture in connection with the patient's worst attack at 2 years 3 months gave a fluid containing 13 WBC/3 2 mm³ (11 mononuclears) and 29 mg% protein. He had no rigidity, no spasticity and no other neurological disturbances.

At later examinations his development has been found to be normal, both mentally and physically.

On the *X*-ray films of his skull, several thin calcifications were seen in the parietal regions.

He has a squint (strabismus concomitans convergens) and his optical discs are pale. Otherwise nothing abnormal was seen at the examination of his eyes, and his vision is to all appearances not impaired.

Serological data:

When the boy was 2 years 3 months samples were taken from the whole family.

	Patient	Mother	Sister, 12 yrs	Brother, 8 yrs	Father
Dye test	1/250	1/50	1/50	neg	neg
CFT	neg	1/7,5	neg	neg	neg

Comment: In this case the diagnosis is less definite than in the previous one. It is obvious from the dye tests that there has been a toxoplasmic infection in the family. The cerebral calcifications are in agreement with a prenatal toxoplasmic infection of the patient. They indicate an early damage of the brain, probably due to toxoplasmosis in this case. It is natural to connect this destruction of brain tissue with the boy's convulsive disorder

Case 4.

S.A.D , a girl born at term, was brought for examination because of suspected mental retardation. At the age of 15 months she was unable to sit without support and had made no efforts to speak Three months later she could sit alone and could say "Mamma"

Her mother, who had given birth to a healthy child a few years earlier, had enjoyed undisturbed health also during her last pregnancy.

Comment: The mother's serological data indicate that she probably suffered from toxoplasmosis in an active and infectious stage during her last pregnancy. Whether her miscarriage three months before her last pregnancy had anything to do with this infection, is difficult to state, although it seems possible.

The child preserved the same dye test titer of 1/250 at 15 months as at 5 months. The serological findings in her blood can consequently not be due to a passive placental transfer of antibodies from her mother. The child must be actually infected with Toxoplasma organisms. When she was 5½ months old her antibody titers were already diminishing, which indicates that she must have been infected some time earlier, probably *in utero*. However, the possibility that she may have aquired her toxoplasmosis after birth, and probably from her mother, cannot be definitely excluded.

Case 2

K.J., a boy born 3 weeks before term, has suffered from convulsions since the age of 6 months. The convulsions engage mostly the left side of the body. They can be fairly well controlled by the use of phenobarbitone (phenemal) in small quantities. He has also a squint. He could walk at the age of 14 months and could speak a few words when 2 years old. He is extremely troublesome to manage and gets angry very easily. He seems slightly mentally retarded.

His mother had not been ill in any way before or during her pregnancy. *She is a trained nurse and worked in an infectious hospital when pregnant.*

A calcification of the size of a pea localized in the middle of the occipital area was seen on the *X*-ray films of the boy's skull.

There is chorioretinopathy of both eyes. The left macula is involved.

In the *EEG* a spike focus can be seen in the right temporal area.

The *ECG* is quite normal and the blood values within normal limits.

Serological data:

The samples were taken when the boy was 3 years old.

Mother		Child	
Dye test	C.F.T	Dye test	C.F.T
1/50	1/7.5	1/50	1/120

Comment: The combination of chorioretinopathy and cerebral calcification make congenital toxoplasmosis probable, while the presence of antibodies in the blood of both mother and child support the diagnosis.

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The child showed no signs of spasticity or rigidity.
An X-ray of her hips revealed nothing abnormal.
She had no cerebral calcifications.
In her left eye she had a chorioretinopathy in the macular region.

Serological data:
Date of birth of child. Oct. 17th, 1953.

Date	Mother		Child	
	Dye test	C F T.	Dye test	C F.T.
1955				
11/1			1/250	1/30
29/1	1/250	1/30		
6/10	1/50	neg	1/50	1/15

Comments: Both mother and child have been infected with toxoplasmosis. The antibody titers in the child's blood are too persistant to have been passively transmitted placentally from her mother during the pregnancy. It seems most likely that the child has acquired her infection in utero. Probably this infection is responsible for her chorioretinopathy and her mental retardation.

SUMMARY

A case of congenital toxoplasmosis is reported, where the patient had as her only complaint a mild convulsive disorder. She had six attacks of convulsions between the age of 2½ and 5 months. Later she has suffered from petit mal of varying frequency. More than a month can pass without any seizures whatsoever. Mental and somatic development normal. The brain shows no calcifications, the electrocardiogram is normal and there is no damage to the eyes.

Three other cases of probable congenital toxoplasmosis are described. The degree of constitutional involvement is different in each case

From earlier reports and from these four cases, it is possible to conclude that congenital toxoplasmosis varies in severity from case to case Relatively benign cases may not be uncommon, if looked for

Department of Pediatrics, The New York Hospital-Cornell Medical Center, New York, U.S.A.

A STUDY OF CONGENITAL TOXOPLASMOSIS

With Particular Emphasis on Clinical Manifestations, Sequellae and Therapy

HEINZ F. EICHENWALD

Nearly twenty years ago, Wolf and his colleagues (1) convincingly demonstrated that the protozoan parasite *Toxoplasma* is the cause of a congenitally acquired infection manifested by chorioretinitis, hydrocephalus, and intracranial calcifications This so-called "classical triad of toxoplasmosis" has since then been described numerous times in the international medical literature, and has come to be accepted as the most common clinical picture presented by the congenital disease

Our animal experimentation (2) however suggested to us that the clinical syndrome described by Dr. Wolf represents only the final stage of this infection, and that toxoplasmosis might take other, more diverse forms, depending on such factors as the time in its intra-uterine development at which the foetus was infected, the rapidity and extent of antibody formation by the mother and its transmission to the foetus and perhaps the virulence of the particular strain of *Toxoplasma* involved

A study[1] was therefore initiated in 1947 to discover what other forms this congenital disease might take, and to determine more precisely the natural history of the infection and its effect on the human host

In order to obtain as unbiased a series as possible, the present study excludes all patients sent to us with a diagnosis of suspected toxoplasmosis from sources other than a group of cooperating hospitals from which patients were referred in a systematic and prearranged manner.

For the initial screening tests, the cooperating institutions supplied us with sera from three groups of infants and their mothers:

The first group consists of infants with any of a variety of signs and symptoms referable to central nervous system disease for which no reasonable etiology could otherwise be established.

The second group consists of young infants with undiagnosed illnesses

1 These investigations were supported in part by Research Grants E-944 and E-998 of the Institute of Allergy and Infectious Disease of the National Institutes of Health

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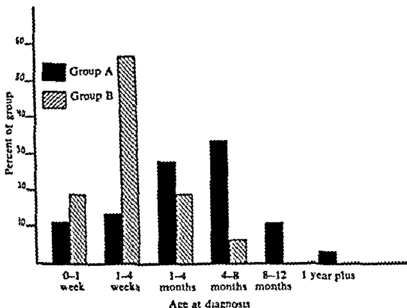


Figure I Age of infants at the time the diagnosis of toxoplasmosis was suggested by serologic tests

mosis, compared to about 1% of infants with more generalized manifestations (Group B). Only a very small, but interesting, fraction of "normal" infants could be shown to have this disease.

The patient's age at the time the diagnosis of toxoplasmosis was made is shown in Figure I Almost three-fourths of the cases of generalized disease (Group B) were diagnosed prior to age one month, but before a similar fraction of infants with primarily neurologic disease had been diagnosed, four months had elapsed. This difference appears to be a reflection of the fact that patients with the generalized disease are more obviously and severely ill during the neonatal period, resulting in prompter and more through diagnostic investigation.

Figure II presents signs and symptoms observed in these patients from the time of birth until the acute phase of the infection was presumably passed The findings are arranged in the order of frequency with which they are found in Group A.

It is obvious that chorioretinitis occurs frequently in either group, although it is not as consistently present as is usually stated. The five most common findings in Group A are chorioretinitis, spinal fluid changes consisting of a pleocytosis and an increased protein content, anemia, convulsions and intracranial calcifications. In Group B, on the other hand, splenomegaly was observed most frequently, followed by abnormal spinal fluid, jaundice, hepa-

characterized by more general findings in whom central nervous system manifestations were either absent or minimal at the time they were referred for study.

The third group is made up entirely of supposedly "normal" newborns.

Toxoplasma dye-test determinations were performed on sera from these infants and their mothers. All sera giving positive results were also examined for the presence of complement-fixing antibodies Criteria used for the serologic diagnosis of toxoplasmosis are those advocated by the *ad hoc* Committee on Toxoplasmosis of the U. S. National Institutes of Health (3). The serologic methods and criteria used in our laboratory have previously been described (4).

Infants with positive serology were throughly examined and retested at age three and six months, at one year and then usually annually thereafter. Only infants whose dye-test antibodies persisted in high titers for six months or more are considered to be serologically proven cases (3), unless they died prior to this time and the parasite was isolated. Each time a follow-up serum specimen was obtained, physical, neurological and developmental examinations were performed, and an interval history taken. All examinations and interviews were conducted by the same physician throughout.

The over-all findings of this study will be presented in the present paper, details of serological titers and their persistence will be discussed in subsequent publications.

The results of the serologic survey of the three groups are presented in Table I.

TABLE I

Number of serologically proven cases of toxoplasmosis found among infants with various clinical conditions

Group	Description	Number of Infants Tested	Number with Serologically Proven Toxoplasmosis	Percent of Group
A "Neurologic" Disease	*Infants with otherwise undiagnosed* CNS diseases in first year of life	2208	108	4 9
B "Generalized" Disease	Infants with otherwise undiagnosed non-neurologic diseases during first two months of life	3284	44	1.3
C "Normal"	Normal infants	5761	4	0 07
	Total ..	11253	156	–

Somewhat over 11,000 infants were tested, a total of 156 confirmed cases of toxoplasmosis were found. Nearly 5% of the infants in Group A, which consists of patients with nervous system disease were diagnosed as toxoplas-

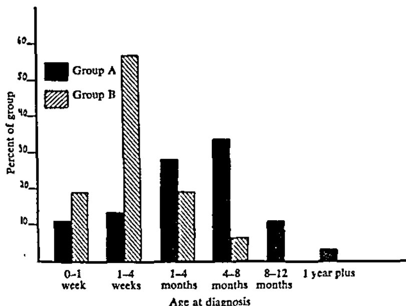


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SIGNS AND SYMPTOMS OCCURRING PRIOR TO DIAGNOSIS OR DURING COURSE OF ACUTE TOXOPLASMOSIS

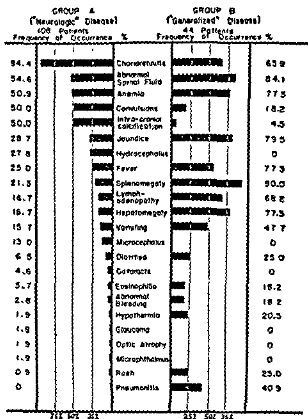


Figure II Symptoms and signs of congenital toxoplasmosis found during the active phase of the disease.

tomegaly, fever, and anemia In this group, such diverse signs as chorioretinitis, vomiting, lymphadenopathy, pneumonitis, and diarrhea were also found with considerable frequency, along with a host of other, less common manifestations Almost every organ system appeared involved, a reflection of the fact that we are dealing with a generalized infection, differing in its clinical picture from that produced by many other infectious agents only in the frequency with which chorioretinitis and perhaps abnormal spinal fluid findings are found.

The symptomatology shown by our group of patients differs in many respects from that recorded in previously published reports. These differences are undoubtedly due to the fact that our patients are drawn from a relatively high. The range of titers shown by Group A is somewhat higher than those unselected group rather than from a limited survey based on patients tested

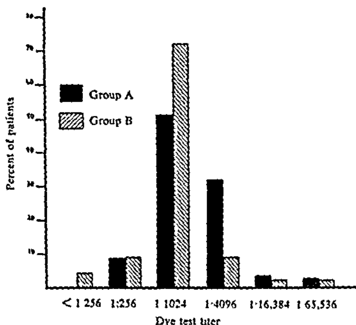


Figure III Range of initial dye-test titers found among infants with congenital toxoplasmosis

only because they showed most of the so-called "classical" signs of toxoplasmosis

The quantitative aspects of the initial dye-test serology on the infants is shown in Figure III. The titers found in sera from these patients are generally of Group B, but the differences are not striking or statistically significant Maternal dye-test antibodies present a very similar pattern The initial complement-fixation tests, on the other hand (Figure IV), show a distinct difference between the two groups, the range of titers of Group A is uniformly higher than that in Group B. Again, the distribution of maternal antibodies is similar to that seen in infants, except that on occasion a mother shows C. F. antibodies while the infant does not (5).

Since it is known that complement-fixing antibodies develop later and rise more gradually than dye-test antibodies (3), one might theorize that these data indicate that the mother and therefore the foetus acquire the infection later in pregnancy in those cases where the infant shows generalized disease (Group B) as compared to those whose nervous system is the major site of involvement.

Some support is lent this theory by the observation that these differences are greatest when complement-fixing titers of the youngest patients in Group A are compared to those of infants of similar ages in Group B. With increasing age, the differences in titer rapidly diminish.

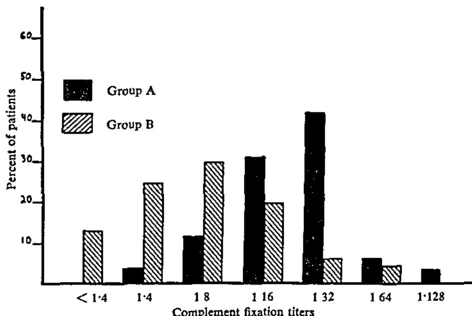


Figure IV. Range of initial complement-fixing titers found among infants with congenital toxoplasmosis

All infants with an established diagnosis of toxoplasmosis have been closely followed since these initial observations. Data on the eventual outcome of the disease are therefore available.

The over-all mortality rate is about 12%. Table II shows that in this respect no significant differences exist between the clinical groups Post-mortem examinations were performed in fifteen infants, in every case the diagnosis of toxoplasmosis was confirmed.

Major sequellae affect the large majority of survivors, only about one infant in ten escapes without serious permanent damage. Table III presents the incidence of sequellae by clinical group, it is of interest that all these conditions are directly referable to central nervous system involvement The over-all figures for Groups A and B are generally similar, although the general trend

TABLE II

Mortality rate among infants with congenital toxoplasmosis

	Number of Patients in Group	Number of Deaths	Percent Mortality
Group A ("Neurologic" Disease)	108	12	11 1
Group B ("Generalized" Disease)	44	6	13.6
Group C (Subclinical Infection)	4	0	0
Total ..	156	18	1[illegible]

TABLE III

Major sequellae of congenital toxoplasmosis found among patients followed four years or more

Condition	Group A		Group B		Group C	
	Neurologic Disease		Generalized Disease		Subclinical Infection	
	Number	Percent	Number	Percent	Number	Percent
Mental Retardation	62	88.6	25	80.6	2	50.0
Convulsions	58	82.9	24	77.4	2	50.0
Spasticity and Palsies	53	75.7	18	58.1	0	0
Severely Impaired Sight	48	68.6	13	41.9	0	0
Hydro- or Microcephalus ...	31	44.3	2	6.5	0	0
Deafness	12	17.1	3	9.7	0	0
"Normal"	6	8.6	5	16.1	2	50.0
Number of Patients in Group	70		31		4	

is for the incidence of each complication to be consistently lower in Group B This would suggest that these infants develop less severe central nervous system lesions during the course of their illness. Two of the four initially normal infants in Group C showed convulsions and mental retardation during infancy and early childhood, while the remaining two are healthy in every respect. These two infants therefore represent cases of subclinical congenital toxoplasmosis.

Since the term "mental retardation" is at best a qualitative one, Stanford-Binet tests were administered to 66 patients. The results are shown in Table IV. Because of the small numbers, the data are not shown by disease groups. The patients tested generally represent those with the best vision and exclude

TABLE IV

Results of Stanford-Binet intelligence tests administered to a group of patients with congenital toxoplasmosis during their fourth to sixth year of life

Intelligence Quotient	Number of Patients	Percent
Over 110	3	5
100–109	4	6
90– 99	2	3
80– 89	8	12
70– 79	11	17
60– 69	14	21
50– 59	10	15
Below 50	14	21
Total	66	100

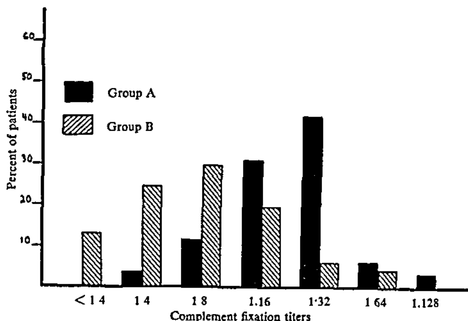


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Condition	Group A Neurologic Disease		Group B Generalized Disease		Group C Subclinical Infection	
	Number	Percent	Number	Percent	Number	Percent
Mental Retardation	62	88 6	25	80 6	2	50 0
Convulsions	58	82 9	24	77.4	2	50 0
Spasticity and Palsies	53	75 7	18	58 1	0	0
Severely Impaired Sight . .	48	68 6	13	41 9	0	0
Hydro- or Microcephalus ...	31	44 3	2	6 5	0	0
Deafness	12	17.1	3	9 7	0	0
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70– 79	11	17
60– 69	14	21
50– 59	10	15
Below 50	14	21
Total	66	100

children institutionalized by their families. Thus, the most severely retarded children are excluded. Despite this fact, almost 60% of this group obtained scores below 70, with only 15% above 90.

We have treated a number of infants with the generalized form of toxoplasmosis, using two different regimens. Both forms of therapy had been shown to be of some benefit in arresting the infection in experimental animals (6, 7).

The first group of 14 patients received a four week course of sulfadiazine along with repeated injections of high-titer anti-*Toxoplasma* sera prepared from humans or rabbits. Later, another group of 10 infants was treated for four weeks with a combination of sulfadiazine and pyrimethamine, both drugs administered in maximum dosage.

Because of the variable clinical course of toxoplasmosis, the immediate effect of either method of therapy is difficult to interpret. It appears, however, that the course of the acute illness is probably shortened by both therapeutic regimens. Spinal fluid findings revert to normal more rapidly than in untreated infants. In three of the treated infants, dye-test antibodies diminished rapidly and were no longer detectable at four years of age. In untreated infants such rapid disappearance of antibodies has not been observed.

The number of treated infants followed long enough for adequate observation of their eventual condition is still too small for final detailed evaluation. It appears at this time however that these methods of therapy are not strikingly beneficial in the prevention of sequellae, although a small favorable effect has not been ruled out.

SUMMARY

The present study indicates that congenital toxoplasmosis is a disease with an extraordinarily wide range of manifestations, so wide in fact that it must be considered in the differential diagnosis of nearly all types of obscure illness occurring during early infancy. The presently available therapeutic methods appear to offer little benefit as far as salvaging a normal child is concerned whether treated or untreated during the acute phase, most infants develop irreversible central nervous system damage.

It seems appropriate therefore to point out that the most profitable direction that research on this disease might take is toward the understanding of its epidemiology so that it may eventually become possible to prevent human infection with the parasite.

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ACQUIRED TOXOPLASMOSIS

From The Toxoplasmosis Department, The State Serum Institute,
Copenhagen, Denmark

CLINICAL AND DIAGNOSTIC ASPECTS OF HUMAN ACQUIRED TOXOPLASMOSIS[1]

J. CHR. SIIM

The first cases of congenital toxoplasmosis were reported in 1923 by the Czech ophthalmologist *Jankû*, and in 1927 by *Torres* from Brazil However, it was not until 16 years later that *Wolf, Cowen & Paige* in the United States, in their now classical study, finally established the toxoplasmic aetiology by isolation of the parasite from a child born with hydrocephalus, cerebral calcification and chorioretinitis. Since then, cases of congenital toxoplasmosis, with the characteristic clinical manifestations, have aroused the interest of paediatricians, neurologists, ophthalmologists, obstetricians and pathologists. Further investigations have revealed an acute active form of the generalized infection in the newborn, with fever, exanthema, meningo-encephalitis, chorioretinitis, jaundice, enlargement of the liver and spleen, gastro-enteritis and a condition resembling erythroblastosis foetalis (*Eichenwald*, 1956). The congenital infection may also run an oligosymptomatic course. In some cases, besides the positive sero-reactions, unilateral chorioretinitis has been the only sign of the disease, and in others slight neurological symptoms have been present, without hydrocephalus, calcification or eye involvement. Thus establishment of the aetiological diagnosis can be extremely difficult, especially in older children.

In addition to the congenital infection, subsequent publications have described rare cases of acquired toxoplasmosis with encephalitis, from one of which the RH strain was isolated (*Sabin*, 1941) A condition resembling typhus was also reported by *Pinkerton & Henderson* in 1941, but intensive clinical research was not initiated until the mainly morphological investigations were supplemented by quantitative serological studies by means of the Sabin-Feldman dye test and the complement fixation test (*Sabin & Feldman*, 1948, *Warren & Russ*, 1948, *Sabin*, 1949)

The combined use of quantitative serological procedures and cultivation trials has finally established the existence of various syndromes of the ac-

1 Aided in part by grants from The King Christian X's Foundation and National Institutes of Health, U.S Public Health Service (Grant No E 1741)

quired toxoplasmic infection characterized in differing variety and incidence by lymphadenopathy, meningoencephalitis, typhus-like exanthema, chorioretinitis or myocarditis. It has also been shown that the toxoplasmic lymphadenopathy occurs quite frequently, in contrast to the relatively rare congenital disease.

In 1950, the first Danish case of *acquired toxoplasmosis with lymphadenopathy* and fever in a child was reported at the VIth International Congress of Paediatrics in Zurich (*Siim*, 1950). The diagnosis was made on the basis of serological examinations carried out routinely on sera from cases with fever of unknown origin It was anticipated that this procedure would reveal cases of acquired toxoplasmosis on the assumption that the acquired infection would also be present in the general population, since it occurred in women who had given birth to children with congenital toxoplasmosis.

Subsequent examination of a number of sera from patients with adenitis of unknown origin, or where the diagnosis was mononucleosis with a negative test for heterophilic antibody revealed a further seven cases with positive sero-reactions (*Siim*, 1951).

At the same time, *Gard & Magnusson* (1950, 1951) in Sweden published a case of lymphadenopathic toxoplasmosis in a pregnant woman, and three cases with positive sero-reactions in patients with lymphadenopathy as the main finding (*Gard*, *1951*, *Magnusson*, *1951*). Thirteen further cases with dye test $\geq$ 1:2000 were reported by *Zeipel et al.* (1951).

In 1952, febrile lymphadenopathy and relative lymphocytosis were demonstrated in a 29-year-old man. A significant rise in both the dye test and complement fixation antibody titres was demonstrated simultaneously with the development of the clinical signs (*Siim*, 1952a).

In the same year Toxoplasma gondii was isolated from a lymph node removed from a 17-year-old girl with non-febrile lymphadenopathy, thus finally proving the toxoplasmic aetiology of the lymphadenopathic disease (*Siim*, 1952b)

At the VIth International Congress of Microbiology in 1953, a further six cases were reported, in all of which the diagnosis was confirmed by isolation of the parasite (*Siim*, *1953*). Similar cases have since been published by *Adamson et al.* (1957), *Armstrong et al.* (1953), *Belfrage et al.* (1957), *Beverley et al.* (1958), *Desmonts* (1956), *Desmonts et al.* (1957), *Garin* (1956), *Grandjean* (1956), *Kayhoe et al.* (1957), *Lelong et al.* (1954), *Siim* (1955, 1956, 1959), and *Siim et al* (1958).

From being an uncommon pathological condition of mainly academic interest, toxoplasmosis has become a disease which is now recognized with increasing frequency in children and adults, both in hospitals and by general practitioners.

Acquired toxoplasmosis has been reported sporadically from various parts of the world, but the disease has not been as widely recognized as appears warranted from the data now available. This is possibly because some countries do not provide a routine laboratory service, and also since many clinicians fail to include this infection in the diagnostic possibilities when dealing with cases of lymphadenopathy or fever of unknown origin, relative lymphocytosis, meningoencephalitis, various eye diseases or myocarditis. At any rate, when carefully looked for, the acquired disease is easily found.

The clinical recognition of so few cases in some countries is rather surprising, since there are certainly good reasons for presuming that the human toxoplasmic infection has a world-wide distribution. Toxoplasmosis has been demonstrated in all warm-blooded animals hitherto examined, both those living wild in nature and in domestic animals and pets in close contact with humans, thus providing sources of infection. Furthermore, serological surveys have shown a high incidence of toxoplasmic antibodies in human populations. Finally, the demonstration of cases of congenital infection proves the existence of the acquired infection in pregnant women at least, since the infection is transferred from mother to foetus.

In order to clarify the clinical and diagnostic aspects of toxoplasmosis so that typical cases are not overlooked, it is essential to provide information in text books and by other means regarding the various clinical manifestations, and also to emphasize the diagnostic laboratory procedures available, the correct way of having them performed, and how to interpret the results. A diagnosis of toxoplasmosis has often been made erroneously and unnecessary treatment instituted, based on sero-reactions carried out with improper techniques or because of misinterpretation of the laboratory results.

A. *Laboratory Diagnosis*

From a clinical point of view the acquired toxoplasmic infection is not characteristic, and in some instances the infection may run an oligosymptomatic or even sub-clinical course. Therefore, the clinical examination and the findings in the clinical-pathological laboratory can merely suggest the possibility that the patient may have a toxoplasmic infection. This can be confirmed in the biological laboratory only by the isolation of Toxoplasma in well-controlled animal experiments, or by the demonstration of a significant rise in the Toxoplasma antibody titre in a comparison of acute and convalescent sera.

The laboratory procedures used in the routine diagnosis of human toxoplasmosis can be divided into direct and indirect methods –

I. *Direct Methods.*
1. Isolation and identification of Toxoplasma.
2. Direct microscopy.

II. *Indirect Methods.*
1. Quantitative serological reactions: –
(a) Sabin-Feldman dye test,
(b) Complement fixation test,
(c) Haemagglutination test,
(d) Precipitation test,
(e) Fluorescence inhibition test.
2. Skin test.
3. Histopathological examination of fixed tissue preparations.

I. *Direct Methods:*

1. Isolation of the protozoan parasite *Toxoplasma gondii* in well-controlled animal experiments is the method of choice and should be attempted whenever possible, even though the procedure is somewhat complicated. The material to be examined may be blood, spinal fluid, urine, exudates, sputum, or tissue removed by biopsy, e g. enlarged lymph nodes, muscle or tonsillar tissue, skin, liver or spleen, or post-mortem specimens. The lymph nodes may be taken surgically in toto, or a needle or Daniels' biopsy may be performed, without risk of the infection being spread

Technique (Siim, 1956) The tissue is minced with scissors and a 20 per cent. suspension made with saline or negative human serum. 0.5 ml of this rather coarse suspension, and not merely the supernatant fluid, is injected intraperitoneally into clean stock mice.

If the material contains Toxoplasma, a latent infection in the mouse often follows the inoculation. This can be demonstrated by the presence of positive sero-reactions in heart blood (*Aagaard*, 1956). The specimens are taken about six weeks after the inoculation, since the Toxoplasma antibodies develop rather slowly after inoculation of a lymph node suspension. Tail blood may be used instead of heart blood, thus allowing the mice to live for further observation.

The serological diagnosis should always be confirmed by the demonstration of Toxoplasma by direct microscopy, either as proliferative organisms in the peritoneal exudate or as pseudocysts in the brain.

By direct microscopic examination of peritoneal exudate, Toxoplasma may be demonstrated in the first or second week after inoculation. In the acute serious cases it is important to attempt demonstration of Toxoplasma in the blood as early as possible so that treatment can be initiated.

It is unnecessary to make sub-inoculations from animals which show

TABLE I

Isolation of Toxoplasma gondii from lymph nodes, muscle and tonsillar tissue in patients with Toxoplasmosis acquisita lymphonodosa

Case No	Sex	Age	Preliminary diagnosis	Sero-reactions Dye test	C F test	Isolation of Toxoplasma	Time between onset and biopsy
1	♀	51	Fever of unknown origin Adenitis	1·6250	1·128	quadriceps muscle	57 days
2	♂	7	Fever of unknown origin Chronic tonsillitis	1:1250	1 128	tonsil	2 months
3	♀	24	Generalized adenitis Toxoplasm.?	1 1250	1 32	axillar lymph node	≧ 6 weeks
4	♀	12	Oligophrenia	1 6250	1 128	inguinal, axillary lymph node	≧ 3 weeks ≦ 3½ months
5	♂	12	Adenitis Infectious mononucl. Toxoplasm Streptococ	1 1250	1 128	cervical lymph node	7½ weeks

negative sero-reactions, since all strains isolated hitherto have caused antibody formation in the first passage.

The mouse is the best experimental animal because it is highly susceptible, and since cases of spontaneous infection seem to occur only with extreme rarity in animals reared in the laboratory. In order to further exclude the occurrence of spontaneous toxoplasmosis, alternate mice are used as controls. Since rabbits and guinea pigs may harbour Toxoplasma spontaneously, these animals cannot be used in isolation trials

The advantages of the intraperitoneal over the intracerebral method are that a larger inoculum can be injected, and a secondary bacterial infection will be better tolerated.

Suspensions of lymph nodes or muscle tissue are bacteriologically sterile. However, when mice are injected with suspensions of tonsillar tissue, the animals should be treated with 100 units G. penicillin and 1 mg. streptomycin for the first two days.

Results (Table 1)· Toxoplasma has been isolated from lymph nodes removed from the occipital and retro-auricular regions, the neck, the axilla

or the groin. The organism has been found even when the biopsy was performed as late as 5½ months after the onset of the disease (*Sim*, 1956).

In 73 per cent. of 38 cases in which the dye test was ≥ 1:1250 and the complement fixation test ≥ 1:8 Toxoplasma could be isolated, but as a general rule not in those cases with dye test titres of less than 1:1250 and with complement fixation titres of less than 1:8 (*Siim*, 1959).

Strains have also been demonstrated in muscle tissue (Case No 1) and in the tonsils (Case No. 2) (*Sim*, 1956).

The strains isolated could be classified as *Toxoplasma gondii* by morphological, tinctorial, pathogenetic and serological studies and could not be distinguished from the RH strains (*Siim*, 1953, 1956, 1959). They have caused antibody formation both spontaneously in man and in experimentally infected animals, and the antibodies could be demonstrated by dye and complement fixation tests using the RH strain as antigen. In protection experiments, rabbits surviving infection with one of the strains isolated were immune to a challenge with the RH strain. In cross neutralization tests using human and hare strains of Toxoplasma as antigen in the dye test, no difference in titre could be demonstrated in various human and animal sera from proven cases. The same results were obtained with cross complement fixation tests, using chorio-allantoic membrane antigens.

Even in the mild cases, the toxoplasmic infection may be generalized. In case No 4 Toxoplasma was demonstrated in lymph nodes removed from two different regions Furthermore, Toxoplasma has been found in muscle and tonsillar tissue from patients with acquired lymphadenopathic toxoplasmosis (Case Nos. 1 and 2).

2. *Direct microscopy* alone is an uncertain method because of the possibility of confusing Toxoplasma with other micro-organisms, e. g. Histoplasma, Cryptococcus (Torula), Sarcocysts, Trypanosoma or Leishmania (*Frenkel*, 1960).

II. *Indirect Methods:*

1. *Quantitative Serological Methods.* Of the indirect methods used for the routine diagnosis, the *Sabin-Feldman dye test* and *complement fixation test* are specific, sensitive and quantitative tests which can be carried out easily and rapidly, and which give reproducible results.

As regards the dye test, the original technique devised by *Sabin & Feldman* has been modified and standardized by *Aagaard* (1956). The method for performing the C. F. test for syphilis has been easily applied to toxoplasmosis, using a chorio-allantoic membrane antigen for both positive and control antigens (*Warren & Russ*, 1948, *Sabin*, 1949).

Results: During acute acquired toxoplasmosis, the dye test usually becomes positive in the second to fourth week and reaches maximum titre values of

1·1000 to 10,000 in the third to fifth week, or later. It remains positive for several years, with slowly decreasing titres. The complement fixation test becomes positive later (*Sabin*) and after one to two months from onset reaches maximum values of 1:100 to 200, which remain thus elevated at least 4–7 months. This test in contrast to the dye test becomes negative earlier, usually two to three years after onset, but slightly positive titre values may be seen for more than six years after the acute stage. However, despite more than six years of observation, the dye test has in no case become negative (*Siim*, 1959).

An evaluation of the serological results may give rise to difficulties because (a) antibodies can be demonstrated in a large number of apparently healthy persons, (b) the reactions are still positive several years after the acute infection, and (c) antibody can be the result of a latent infection. It is therefore obvious that the demonstration alone of positive titres in an infectious illness of uncertain aetiology can by no means be taken as proof that the disease is actually caused by Toxoplasma Only if a significant rise in titre is demonstrated at the same time as the clinical symptoms and signs develop can the toxoplasmic aetiology be proven serologically On the other hand, repeated negative dye tests seem to exclude the possibility of an acute toxoplasmosis.

The haemagglutination test (*Jacobs & Lunde*, 1957), the precipitation test (*O'Connor*, 1957) and the fluorescence inhibition test (*Goldman*, 1957) have given promising results, also from a theoretical point of view. However, more extensive experience is required before the value of these tests in routine diagnosis can be determined.

2. The *Skin Test* (*Warren & Russ*, 1948, *Frenkel*, 1948, *Feldman & Sabin*, 1949) is a qualitative reaction and therefore of only limited value in the diagnosis of acute cases of acquired toxoplasmosis. However, it is of importance in the diagnosis of chronic cases and in the epidemiological study of larger population groups.

3 *Histo-Pathological Examination* Microscopical examination may reveal a reticulum cell hyperplasia with islands of reticulum cell scattered over the whole of the section, and also chromatolytic changes. Although the histological picture is presumably not pathognomonic, it is so characteristic that when demonstrated in a lymph node or a tonsil biopsy, it will justify the serological tests (*Alexander*, 1955; *Bang*, 1953, 1957; *Landau*, 1951, *Piringer-Kuchinka*, 1958; *Robb Smith*, 1958; *Saxen et al*, 1958, *Siim*, 1951, 1952, *Stanton et al*, 1952, *Wahlgren*, 1951, 1958). However, further studies of lymph nodes from parasitologically verified cases and from patients with lymphadenopathy of other origin are still required.

Isolated Toxoplasma has not been found microscopically with certainty in human toxoplasmic lymph nodes, and the occurrence of pseudocysts has

hitherto been demonstrated in two cases only (*Stanton & Pinkerton; Wahlgren*).

B. *Clinical Manifestations*

A classification of the various clinical manifestations of acquired toxoplasmosis may be made according to the following main symptom complexes, which are listed in order of importance, and which may also occur in various combinations (*Siim*, 1955, 1956): –

(a) *Acquired Toxoplasmosis:*
1. *Toxoplasmosis acquisita lymphonodosa,*
2. *Toxoplasmosis acquisita cerebrospinalis,*
3. *Toxoplasmosis acquisita exanthematica,*
4. *Toxoplasmosis acquisita ophthalmica,*
5. *Toxoplasmosis acquisita myocardialis.*

(b) *Congenital Toxoplasmosis.*

This clinical division of acquired toxoplasmosis into main groups also seems to be of didactic value However, as Toxoplasma may invade almost every tissue, symptoms and signs may be expected in other organs, the disease thus becoming of interest to almost all medical fields.

Since the majority of verified cases hitherto published belong to the acquired toxoplasmic lymphadenopathy group, a description of the clinical manifestations of this form of the disease is included here.

According to nosographical studies (*Siim*, 1956, 1959) toxoplasmosis with lymphadenopathy can be divided into three sub-groups: –

1. A *febrile* form, in which the onset can be either acute with chills, or gradual, and in both of which the temperature is increased to 38°–40° C. for three to six weeks or longer.
2. A *non-febrile* form, in which the enlarged lymph nodes are discovered by the patients themselves or by members of their family.
3. A *sub-clinical* form, in which the lymphadenopathy is demonstrated by the physician either during routine examination of apparently healthy persons or during examination of members of the family in which a case of acquired toxoplasmosis has already occurred

The following cases will illustrate these three forms of lymphadenopathic acquired toxoplasmosis.

Case Nos. *1* and 2 are characteristic of the febrile form.

Toxoplasmosis Acquisita Lymphonodosa Febrilis.

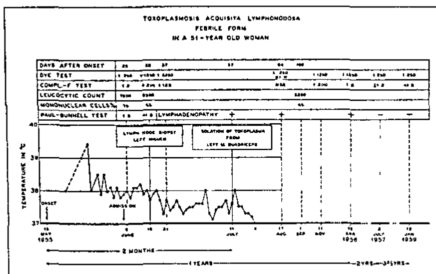


Fig. 1

Case No. 1 (Fig 1)

History. A 51-year-old female office worker (Medical Department, Sundby Hospital, Copenhagen Case No. 1217/1955) After having been previously healthy, the patient was suddenly taken ill on May 15 or 16, 1955, with chills, slight sore throat and malaise She continued her office work for five days, but afterwards stayed in bed Despite treatment with penicillin, the temperature remained elevated (between 38° and 39 4° C) and she was admitted to the hospital 24 days later with the diagnosis Fever of unknown origin. The appetite had been poor for a week, but there had been no loss of weight There had been no headache, disturbance of vision, catarrh, exanthema, itching of the skin, cardiac symptoms, muscular pains, symptoms of the gastro-intestinal or urinary tract, nor had there been stitch under the left rib

On admission, on June 8, the general condition was unaffected The temperature was 37 9° C, pulse rate 70 per minute. Apart from slight redness in the throat, nothing abnormal was found. Two days later, non-tender lymph nodes up to hazel nut size were found on the neck, and smaller lymph nodes in the axillae and groins The lymph nodes were firm, well-defined and did not adhere to the covering skin or underlying tissue The skin was normal There was no catarrh and no cardiac signs The breasts were normal. There was no enlargement of the liver and spleen, and the reflexes were normal

The *preliminary diagnosis* was Fever of unknown origin, acute pharyngitis, and cervical and axillary lymphadenopathy

Course· Histological examination of a cervical lymph node removed eight days after admission suggested the possibility of reticulo-sarcoma

Since lymphocytosis was demonstrated and the heterophile antibody test was negative, sero-reactions for toxoplasmosis were carried out thirteen days after admission These showed a titre of 1 6250 in the dye test and 1·128 in the complement fixation test

Despite an increased temperature of between 38° and 39 4° C. for more than 35

days, the general condition was remarkably unaffected, and treatment with penicillin, tetracycline and sulphadiazine had no effect.

A significant rise in the Toxoplasma antibody titres was demonstrated, and the serological diagnosis was later verified by the isolation of Toxoplasma from muscle tissue removed from the left quandriceps muscle 57 days after onset. (Fig 1). Electromyography showed a significant decrease in the action potential duration consistent with a *myogenic affection*

The patient was discharged after 40 days in the hospital Recovery was complete After a month's convalescence, during which the patient remained very tired, she resumed her office work.

On follow-up six months after onset of the disease, she was well. Lymph nodes the size of peas could be demonstrated in the axillae only The dye test was 1:1250 and *the complement fixation test 1 8(16) Five months later the condition was unchanged* Two years after onset there was no lymphadenopathy. The dye test titre had decreased to 1 250 and the complement fixation test was $\leq$ 1 2. Three-and-two-thirds years later, she was still fit and well There were no eye symptoms and no lymphadenopathy. The dye test was still positive (1.250) but the complement fixation test had become negative (< 1 2).

Treatment She was confined to bed for 40 days. Penicillin, tetracycline and sulphadiazine were without effect.

Laboratory examination On June 9 and July 7, haemoglobin values were 82 and 87 per cent and sedimentation rates 50 mm and 35 mm (*Westergren*) respectively. On June 9 and June 15 the leukocyte counts were 7,600 and 9,500 respectively Differential counts on June 11 and June 15 neutrophils – unsegmented in respective order. 2 and 1 per cent, *segmented 21 and 35 per cent., eosinophils 0 and 1 per cent; basophils 0* and 0, monocytes 0 and 3 per cent., small lymphocytes. 30 and 45 per cent; large lymphocytes 45 and 15 per cent.

The urine contained no albumin

The thymol turbidity tests on June 25 and July 8, 1955 and January 17, 1956 were respectively 0 32, 0.32 and 0 21, the plasma bilirubin index (*Meulengracht*) was normal *and the Takata-ara test was negative The heterophile antibody tests on June 6 and 16 were* negative (1 8 and < 1.8) The Wassermann reaction, cold agglutination test, Weil and Widal tests, and the agglutination test for haemolytic streptococci were negative The antistreptolysin titre was normal

X-ray examination of the chest showed nothing abnormal

The *ophthalmoscopic examination* and *electrocardiogram* were normal

Electromyography on February 16, 1956, showed decreased action potential duration [illegible] 1957, [illegible] atus

(Erna Christensen)

Isolation of Toxoplasma On July 11, 1955, 57 days after onset of the disease, Toxoplasma was isolated from a muscle biopsy of the left quadriceps

Summary.

A 51-year-old woman was suddenly taken ill with fever of unknown origin, and with chills, slightly sore throat and malaise.

On examination at the hospital 24 days later, the general condition was unaffected. Generalized lymphadenopathy, an *increased sedimentation rate,*

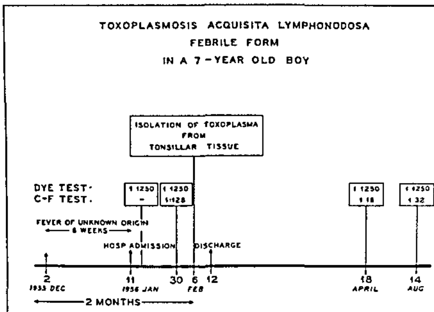


Fig 2

lymphocytosis and a positive thymol turbidity test were demonstrated. The preliminary diagnosis was: fever of unknown origin; acute pharyngitis; cervical and axillary lymphadenopathy; reticulosarcoma. Because of the lymphocytosis and a negative Paul Bunnell test, sero-reactions for toxoplasmosis were carried out with positive results and the diagnosis was verified by the isolation of Toxoplasma from the left quadriceps 57 days after onset Histological examination of the biopsy revealed myositis, and electromyography carried out later showed a decreased action potential duration Recovery was complete. On examination two years and three-and-two-thirds years after onset, there were no enlarged lymph nodes, the dye tests were 1 250 and the complement fixation test $\leq$ 1·2 and $<$ 1:2, respectively.

Case No 2 (Fig 2)

History· A 7-year-old schoolboy (Queen Louise's Children's Hospital, Copenhagen. Case No. 62/1956) during the past year had had febrile episodes (38°–38 6° C) lasting for a week about once monthly, and often with signs of a cold Six weeks before admission he suddenly developed a temperature of 39° C and purulent coryza Despite being kept in bed for about a month and treatment with penicillin, the temperature remained elevated (38° C) and the patient was therefore hospitalized with a diagnosis of fever of unknown origin, sepsis, and endocarditis lenta There had been no headache, disturbance of vision, cough, exanthema, symptoms from the gastrointestinal tract, stitch under the left rib, muscular or neurological symptoms

On admission· (January 11, 1956), the general condition was unaffected The tem-

perature was only slightly increased (37.5°–37.9° C.) and soon became normal. Chronic tonsillitis as well as cervical lymphadenopathy and lymphocytosis were present. There was no generalized lymphadenopathy, no exanthema, no respiratory, cardiac, or neurological signs, and no enlargement of the liver or spleen. The teeth were normal. The preliminary diagnosis was: Fever of unknown origin, chronic tonsillitis.

Course: On routine examination six and nine days later, the Sabin-Feldman dye test was 1:1250 and complement fixation test 1:28. Repeated clinical examination now revealed generalized lymphadenopathy with tender, firm, discreet lymph nodes the size of hazel nuts to walnuts in the occipital region, on the neck, in the axillae and in the groins. No enlargement of the spleen could be demonstrated.

The serological diagnosis was confirmed by isolation of Toxoplasma from tonsillar tissue removed about 2 months after the onset of fever.

There were no complications from the eyes, brain or heart.

Treatment consisted in confinement to bed, penicillin and the Finsen light.

The patient was discharged after 32 days in hospital fit and well, except for pronounced tiredness which persisted for about four months. On follow-up 9½ months after onset of the fever, generalized non-tender lymphadenopathy was still present. The dye test was: 1:1250 and the complement fixation test 1:32.

Laboratory examination: Haemoglobin values, sedimentation rate, leucocytic count, thrombocytic count and thymol turbidity test were normal. The differential count on January 13 showed 70 per cent. mononuclear cells (leukocytic count 10,100). The Wassermann, Paul-Bunnell and thymol turbidity and cold agglutination tests were negative. Antistreptolysin titre normal.

Electrocardiogram, X-ray examination of chest and skull, *electromyogram* (April 1956) and *ophthalmoscopy* were normal.

Examination for Toxoplasmosis: On January 20 and August 14, 1956, the dye test titres were respectively 1:1250 and 1:1250, and the complement fixation test titres 1:128 and 1:32 respectively.

Isolation of Toxoplasma: On February 6, 1956, two months after onset, 1.0 ml. of a 20 per cent. suspension of tonsillar tissue was injected intraperitoneally into each of 23 mice, 21 of which later showed positive dye tests.

Summary.

A 7-year-old boy was hospitalized because of fever of unknown origin of about six weeks' duration. On examination, chronic tonsillitis, cervical lymphadenopathy – and six days later generalized lymphadenopathy – and lymphocytosis were found. Toxoplasma was isolated from his tonsillar tissue removed two months after onset.

On follow-up 9½ months after onset, he was well, though a generalized non-tender lymphadenopathy was still present. The dye test was 1:1250 and the complement fixation test 1:32.

The *non-febrile form* is illustrated by Case Nos. 3, 5 and 6.

In Case No. 3 the patient became pregnant after the onset of the acquired toxoplasmosis when the Toxoplasma antibody titre was still high. The child was normal. However, in Case No. 6 the toxoplasmic infection was acquired after the patient had become pregnant, and the infection was transferred to the foetus.

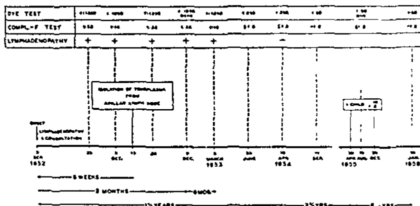


Fig. 3

Toxoplasmosis Acquisita Non-febrilis

Case No 3 (Fig. 3).

History: 24-year-old housewife (Medical Out-Patients' Department, Rigshospitalet, University of Copenhagen. Case No 3729/1952)

The patient consulted her doctor on September 2, 1952, because of non-tender nodes on both sides of the neck. She had previously been in good health, but during the past month or so had been unduly tired, with non-characteristic headache and dyspnoea on exercise. However, she had been able to carry out her work. She complained also of her hair falling out.

On examination, a generalized lymphadenopathy was demonstrated, with firm, non-tender lymph nodes Since the doctor had previously seen cases of acquired toxoplasmosis, sero-reactions for toxoplasmosis were carried out on September 25 with positive results (dye test 1 1250, complement fixation test 1 32), and the patient was referred to the Medical Out-Patient's Department on October 6 with the diagnosis Generalized adenitis, observation for toxoplasmosis; pityriasis simplex

The patient had slept well and her appetite was good There had been no disturbance of vision, dizziness, catarrh, exanthema, itching of the skin, pains in the joints or muscles, symptoms from the gastro-intestinal or neurological system, stitch under the left rib, or loss of weight. There had been no chills but some perspiration. The patient did not think that her temperature had been increased

On examination, the general condition was unaffected Enlarged lymph nodes could be demonstrated in the occipital region, on the neck, in the axilla, in the left cubital region and in the groins. The lymph nodes, which were the size of peas to hazel nuts, were non-tender, smooth, well-defined, firm and covered by normal skin. There was no exanthema. Examination of heart and lungs was normal The breasts were normal and there was no enlargement of liver and spleen. Reflexes were normal.

The *preliminary diagnosis* was: Generalized adenitis, acquired toxoplasmosis

Course· On October 13 a lymph node was removed from the left axilla and Toxoplasma was isolated after intraperitoneal injection into clean mice (Table 1)

No treatment was instituted

On follow-up three and six months after the onset, the patient was tired but carried out her normal work There was no change in the enlarged lymph nodes The dye test values for the successive tests of the two follow-ups were 1:1250(6250) and 1.1250 and the complement fixation values 1 32 and 1:16.

On follow-up 1½ years after onset, no enlarged lymph nodes could be palpated, the dye test was 1 250 and the complement fixation test ≦ 1.2

Three years after onset the patient was still healthy There had been no symptoms from the eyes and swollen lymph nodes could not be demonstrated. The dye test was 1 50(250) and the complement fixation test ≦ 1·2

On April 30, 1955, a healthy child was born. The dye test and complement fixation tests on the child were negative six months later. Ophthalmoscopic and X-ray examinations of the skull showed nothing abnormal.

At the last follow-up in January 1959, six-and-one-third-years after onset, the mother and child were both healthy. The mother's dye test titre had fallen to 1 50 and the complement fixation test had become negative ($<$ 1.2)

Laboratory examinations Haemoglobin value, sedimentation rate, leukocyte count and differential counts were normal.

The urine contained no albumin

The Wassermann reaction, Paul-Bunnell test and thymol turbidity test (0 13) were negative

X-ray examination of skull and chest was normal.

Ophthalmoscopy· normal

Electrocardiogram normal.

Summary.

A 24-year-old housewife accidentally discovered indolent lymph nodes on both sides of the neck During the previous months she had complained of tiredness, headache and dyspnoea. The doctor's examination revealed generalized lymphadenopathy and positive sero-reactions for toxoplasmosis (dye test 1:1250, complement fixation test 1:32).

On examination in the Out-Patients' Department, except for demonstration of generalized adenopathy, clinical and laboratory examinations showed nothing abnormal. The serological diagnosis was verified by the isolation of Toxoplasma from a lymph node removed from the left axilla six weeks after the first consultation

The course was uncomplicated. Four years after onset, a healthy child was born.

About six years after the recognition of the toxoplasmic infection the dye test was still positive, 1.50, while the complement fixation test had become negative.

In the *sub-clinical form*, the lymphadenopathy is revealed accidentally on routine examination (Case No. 4). *This group comprises also cases of preg-*

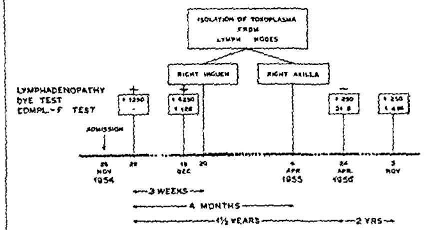


Fig 4

nant women, the majority of whom have apparently been healthy without any history of definite illness, previous to or during pregnancy, but who, despite this, give birth to children with congenital toxoplasmosis.

Toxoplasmosis Acquisita Lymphonodosa Sub-clinica

Case No 4 (Fig 4)

History (The Children's Hospital, University of Copenhagen, Case No 1169/1954) A 12-year-old girl, daughter of a labourer, apparently somatically healthy, was hospitalized for observation because of oligophrenia of about nine years' duration There had been no previous acute infection or trauma of the head Except for minor catarrhal disorders, the patient had not been ill during recent years and she had had no attacks of fever There had been no headache, disturbance of vision, dizziness, sore throat, symptoms from the respiratory or digestive tract, and no pains in the muscles

On admission, on November 26, 1954, the general condition was unaffected, there were no complaints and the temperature was normal On the neck, body and proximal parts of the thighs, remains of exanthema (resembling pityriasis rosea) were seen Except for a left-sided strabismus (observed when she was 5 years old), no other signs were present, particularly no respiratory, cardiac, muscular or neurological signs There was no enlargement of liver or spleen

Diagnosis The preliminary diagnosis was oligophrenia.

Course Since a routine examination for toxoplasmosis was carried out at that time on all neurological and psychiatric patients, positive sero-reactions were revealed on November 29, the dye test titre being 1 1250. At the same time, on renewed examination

non-tender lymph nodes the size of cherries were found in both axillae, and lymph nodes the size of peas in the groins There was no adenitis in the neck and no swelling of the cubital lymph nodes. The enlarged lymph nodes were smooth, hard, mobile and covered with normal skin In order to confirm the serological diagnosis, a lymph node was removed from the right groin on December 20, and 3½ months later a lymph node was also removed from the right axilla In both cases Toxoplasma was isolated by injecting a lymph node suspension intraperitoneally into clean mice (Table 1)

No treatment was given.

The course was uncomplicated. The enlarged lymph nodes were still present four months after the serological diagnosis was made but had disappeared about one year later. One-and-a-half-years and two years later the dye test and complement fixation test titres had fallen to 1 250 and 1 250 and ≦ 1 8 and 1 4(8), respectively (Fig 4)

Laboratory examination The haemoglobin value and sedimentation rate were normal On December 8, 1954, the leukocyte count was 6,440 Differential count neutrophils, unsegmented, 1 per cent , segmented 44 per cent.; eosinophils 2 per cent ; small lymphocytes 48 per cent ; monocytes 5 per cent

The urine contained no albumin.

The intradermal tuberculin test, Wassermann reaction and Paul-Bunnell test were negative.

X-ray of the skull showed no calcification and *X-ray of the chest* was normal.

Electro-encephalogram was abnormal, with a focus in the right occipital region Spinal tap was not performed (In 1956 a spinal tap was normal)

Ophthalmoscopy nothing abnormal.

Electromyography on May 3 and November 8, 1956 (*F. Buchthal*) showed decreased action potential duration (right rectus femoris 6 7 ms (58 potentials), right biceps femoris 6 4 ms (31 potentials))

Muscle biopsy on May 8, 1956 from the right rectus femoris Toxoplasma could not be isolated *Microscopical examination* Nothing abnormal (*Erna Christensen*)

Summary.

A 12-year-old girl, apparently somatically healthy, was admitted to the hospital for observation for oligophrenia of about nine years' duration. There were no clinical findings on admission. On routine examination for toxoplasmosis, positive sero-reactions were demonstrated (dye test 1.6250, complement fixation test 1 128). Swollen, but non-tender, lymph nodes were then found in the axillae and groins The remains of exanthema, lymphocytosis, abnormal electro-encephalogram and decreased potential duration on electromyography were the other findings. The diagnosis of toxoplasmosis was confirmed by the isolation of Toxoplasma from a lymph node biopsy from the right inguen and also, 3½ months later, from a lymph node from the right axilla.

The course was uncomplicated.

Symptomatology.

The following is a brief survey of the symptomatology of toxoplasmosis with lymphadenopathy, based upon observation of parasitologically verified

cases. On account of the relatively small number of cases observed, the morbidity, age and sex distribution and frequency of the various symptoms and signs have yet to be clearly defined.

Incidence: Preliminary investigations in Denmark have shown that the lymphadenopathic form is the one which occurs most frequently in both children and adults of both sexes. In a series consisting of 40 patients with lymphadenopathy of unknown origin, 13 per cent. had serologically verified toxoplasmosis, while 15 per cent. had other diseases of well-established aetiology, i. e. Hodgkin's disease, lymphosarcoma, metastases or tuberculosis (*Siim*, 1959).

Incubation. The incubation period cannot be estimated in the spontaneous cases, since the source of the infection is not known However, in four laboratory cases, in which infection occurred as the result of a finger being pricked with an infected needle, the incubation periods were three, five to seven, eight and nine days respectively (*Strøm*, 1951, *Kayhoe et al*, 1957; *Soestbergen*, 1957; *Beverley et al.*, 1955).

Onset: The onset may be acute with rise in temperature to 39–40° C., accompanied in some cases by a chill. Headache and malaise may be present, but on the whole the general condition is unaffected Moderate sore throat, gastro-enteritis, aches or coryza have sometimes been observed. In other cases the onset is gradual.

The non-febrile form is symptomatically an extremely mild disease in which swollen, tender or indolent lymph nodes are discovered by the patients themselves, who have carried out their work despite the disease

Lymphadenopathy: Lymphadenopathy and increased temperature are the main symptoms. Swollen lymph nodes, the size of peas to hazelnuts or walnuts, are found in all the patients They may be tender but may also – even in acute cases – be indolent They are smooth, firm, of uniform consistency, and well-defined, without oedema, and not adhering to the covering skin or underlying tissues. The skin is never red or hot, and petechiae or itching of the skin, suppuration of the lymph nodes or formation of fistulas have never been observed.

The lymphadenopathy is usually generalized and it is seldom that lymph nodes are affected in one region only Swollen lymph nodes may be present in the hilus of the lungs. It is characteristic that when the lymph nodes have reached their maximum size in the acute stage of the disease, the swelling remains stationary for a long period and then decreases slowly. Usually the lymph nodes remain enlarged for six to twelve months or more.

There seems to be no direct relationship between the size of the lymph nodes and the severity of the case, the degree of fever, or the positivity of the sero-reactions. On the contrary, in cases with high fever the lymphadenopathy may not be remarkable.

Enlargement of the spleen is seldom demonstrable on palpation, but may be found by X-ray examination (*Siim & Nissen*, 1958).

The *temperature* is usually increased for a long period, in some patients for five to seven weeks. Relapses have not been seen.

Other signs· Exanthema is only rarely observed, even though tests for isolation of the agent have shown that the infection has been generalized. Noncharateristic *muscular pain* may be present, but such signs may be absent even in cases where Toxoplasma has been isolated from muscle tissue.

Clinical evidence of pulmonary involvement has not been found. Signs related to the *heart* or *eyes* have been seen in only about 1 per cent. of the cases.

On *laboratory examination* the haemoglobin value is normal or only slightly reduced. The leukocyte and platelet counts are normal. The sedimentation rate in the febrile cases is often moderately increased but in the non-febrile cases is usually normal.

The leucocytic count is normal, but relative lymphocytosis (45–70 per cent.) is found with large atypical lymphocytes resembling the McKinlay cells.

The thymol turbidity test is often positive in the febrile cases (*Siim*, 1952a). However, the liver function tests are normal and the Paul-Bunnell test negative

Course· The course is usually benign and the patients make complete recovery. Complications from the eyes or heart have hitherto been rarely observed, and it has not been possible to establish their toxoplasmic aetiology. Despite observation of the primary uncomplicated cases for several years, recurrence or signs of involvement of the central nervous system, the eyes or the heart have not been found as yet. The enlarged lymph nodes, however, persist for 6–12 months or longer, and in some cases, convalescence is accompanied by pronounced tiredness over a period of months, perhaps because the muscles are affected as part of the generalized infection (*Siim*, 1956). In preliminary investigations with *Buchthal* and *Erna Christensen*, electromyography has in some cases shown a significant decrease in potential duration and myositis has been demonstrated microscopically (Case No. 1).

Differential Diagnosis. The clinical manifestations of acquired toxoplasmosis with lymphadenopathy are by no means characteristic for this infection alone, and a typical exanthema or characteristic temperature curve are not seen It is evident, therefore, that other diseases with lymphadenopathy or fever can be mistaken for toxoplasmosis, and for the same reason the diagnosis of this quite oligosymptomatic infection calls for the alertness and knowledge of the physician

The clinical demonstration of generalized lymphadenopathy, with firm,

smooth, discreet nodes covered by normal skin, and the unaffected general condition despite high fever, can cause one to suspect the diagnosis. This can be supported by the finding of relative lymphocytosis with atypical lymphocytes, a positive thymol turbidity test and histological changes in the lymph nodes. However, only a significant rise in the serological titres or isolation of Toxoplasma can finally verify the diagnosis. Since the demonstration of even strongly positive sero-reactions only justifies the conclusion that the patient has or within the past year has had a toxoplasmic infection, the possibility of other diseases must be eliminated

During observation of a great number of cases, the following diseases were considered in differential diagnosis

1. *Diseases with enlarged lymph nodes*·
   (a) *Infectious*· Infectious mononucleosis, streptococcosis, tuberculosis, syphilis, mumps, histoplasmosis or cat-scratch disease
   (b) Boeck's sarcoid.
   (c) Malignant lymphoma (Hodgkin's disease, Brill Symmers' disease, reticulo-sarcoma), leucosis, or tumour of the salivary glands.
   (d) Metastases (from breasts or mediastinum).
2. Diseases with lymphocytosis.
3. Fever of unknown origin

In the rather rare cases in which lymph nodes are enlarged in only one region, malignant diseases should be excluded Since verified toxoplasmosis and malignant tumour have been observed in the same patient (*Siim*, 1956; *Grandjean*, 1956) a biopsy should always be performed in such cases.

The differential diagnosis between mononucleosis and toxoplasmosis may be difficult, but in the typical cases a detailed anamnesis and thorough clinical and laboratory examinations will often give a strong suggestion of the correct diagnosis.

In differentiating between toxoplasmosis and the febrile form or atypical slight cases of infectious mononucleosis, serological examination is of particular importance, since clinical and serological observations, together with absorption experiments, do not support the assumption of a common antigen (*Siim*, 1959)

Occasionally, toxoplasmosis and mononucleosis have been diagnosed in the same patient at different times (*Siim*, 1952c, 1956). In Case No. 5 in which positive toxoplasmic reactions were still present after acquired toxoplasmosis, infectious mononucleosis occurred 1¾ years later and did not give rise to an increase in the toxoplasmic antibody titre, and in another patient, the toxoplasmic infection did not cause a rise in the heterophilic antibody titre.

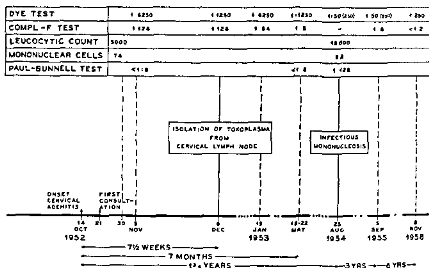


Fig 5

Case No 5 (Fig 5).

History A 12-year-old school-boy (The Finsen Institute, Paediatric Out-Patient Department, Case No 26566, and Blegdams Hospital, Epidemic Hospital of Copenhagen, Case No. 1137/1941) previously in good health observed by accident about October 14, 1952 a non-tender swelling on both sides of the neck. There were no other symptoms, no fever, and the general condition was completely unaffected As the swelling persisted, the mother consulted the physician a week later because of fear of a malignant condition

On examination on October 21 and and November 13, the general condition was unaffected and the temperature normal Except for non-tender lymph nodes the size of beans on both sides of the neck, there were no other signs, particularly no exanthema, no respiratory, cardiac or neurological signs There was no enlargement of the spleen or liver. The preliminary diagnosis was Cervical adenitis; infectious mononucleosis; toxoplasmosis, streptotococcal infection

Because of the demonstration of lymphadenopathy and relative lymphocytosis, seroreactions for toxoplasmosis were carried out on November 3, with positive results (dye test 1 6250, complement fixation test 1 64(128)) The diagnosis was confirmed by the isolation of Toxoplasma from a lymph node removed 7½ weeks after onset from the right side of the neck.

The course was benign The swelling of the lymph nodes was still present seven months after onset at which time the dye test was 1·1250 and the complement fixation test 1.8.

Treatment Except for universal Finsen light (carbon arc), no therapy was instituted.

Laboratory examination The haemoglobin value and leukocyte count were normal The differential count showed 74 per cent. atypical mononuclear cells (leukocytic count

5,000). The thymol turbidity test, Wassermann reaction and Paul-Bunnell test were negative The antistreptolysin titre was increased (1.400).

Isolation of Toxoplasma: On December 6, 1952, 7½ weeks after onset, Toxoplasma was isolated from a cervical lymph node.

About one-and-three-quarter-years after onset he was admitted to the Epidemic Hospital with a clinically and haematologically typical infectious mononucleosis with generalized lymphadenopathy, positive Paul-Bunnell test (1.128, absorption ad modum Davidsohn typical) The leukocyte count was 18,000 with large lymphocytes 90 per cent., small lymphocytes 1 per cent, and monocytes 1 per cent. The dye test was 1:50 (250) and a month later 1·50, while the complement fixation test was unreadable

On follow-up three and six years after onset, the patient was fit and well. There was no lymphadenopathy. The dye tests were 1 50(250) and 1 250 respectively, and the complement fixation tests 1 8 and negative (< 1 2) respectively.

Summary.

A 12-year-old schoolboy accidentally discovered non-tender nodes on both sides of the neck The physician found cervical adenitis and relative lymphocytosis and on preliminary diagnosis suggested Adenitis, infectious mononucleosis, toxoplasmosis, streptococcosis.

The dye test was 1:1250 and the complement fixation test 1:64(128). The serological diagnosis was verified by the isolation of Toxoplasma from a lymph node removed from the right side of the neck about 7½ weeks after onset.

About 1¾ years after onset he had typical infectious mononucleosis. The dye test was 1·50(250) and the titre did not rise during the infectious mononucleosis. Six years after onset, the dye test was 1 250 and the complement fixation test negative (< 1.2)

When considering the differential diagnostic possibilities, it is important to keep toxoplasmosis in mind Table 2 lists the various conditions in which a serological examination for toxoplasmosis is indicated (Siim, 1956, 1959)

Table 2 *Conditions in which serum testing for toxoplasmosis is indicated*

(a) Fever of unknown origin,
Lymphadenopathy,
Infectious mononucleosis (Paul-Bunnell test negative),
Relative lymphocytosis.

(b) Encephalitis,
Serous meningitis.

(c) Exanthema (resembling typhus).

(d) Chorioretinitis

(e) Myocarditis.

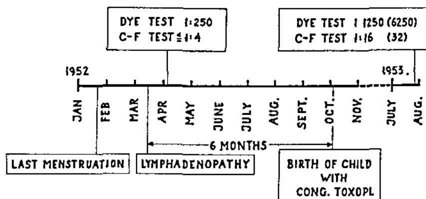


Fig 6

An early diagnosis either based on the demonstration of a significant rise in serological titres or by the cultivation of Toxoplasma from the blood is of value in the severe cases so that treatment can be commenced immediately. For pregnant women the diagnosis is decisive when the pregnancy may have to be terminated.

C. *Toxoplasmosis and Pregnancy*

Cases in which pregnancy and toxoplasmosis occur simultaneously can be divided into two main groups, each with different prognosis and treatment.

1. To the first group belong women who have no Toxoplasma antibodies when they become pregnant and who during pregnancy acquire acute toxoplasmosis. Here haematogenous dissemination may give rise to foci in the placenta, from which the infection may be transmitted to the foetus.

In the following Case No. 6 (Fig. 6) a non-febrile lymphadenopathy was diagnosed in a pregnant woman who gave birth to a child with toxoplasmosis. The results of the serological examinations carried out later made the diagnosis of acute toxoplasmosis in early pregnancy a probable one

Case No. 6.

History· 29-year-old housewife (Finsen Institute, Department for Surgical Tuberculosis. Case No. 35028). Fig 6. Seven to eight weeks after the last menstruation, the patient observed enlarged, non-tender nodes on the right side of the neck. There was no catarrh, angina or fever

On examination three weeks later, lymph nodes the size of peas to beans were demonstrated in the right retroauricular region, behind the right sternocleidal muscle, and in both axillae

Laboratory examination showed a total leucocyte count of 5,400 with 39 per cent. mononuclear cells The antistreptolysin titre was normal and the Paul-Bunnell test negative

Six months later a child was born about three weeks before term and weighing 2850 gr At the age of about two months hydrocephalus was seen and subsequent ophthalmoscopic examination revealed chorioretinitis

The blood sample, which was sent to the State Serum Institute in the second to third month of pregnancy for examination for heterophilic antibody and which had subsequently been stored at — 30° C, was then examined for toxoplasmic antibody. The dye test was 1 250 and the complement fixation test ≦ 1 4, while the titres in a blood sample taken about nine months after birth had risen to 1 1250(6250) and 1 16(32) respectively (Fig 6)

Summary.

A 29-year-old pregnant woman had generalized lymphadenopathy with slight positive sero-reactions for toxoplasmosis seven to eight weeks after the last menstruation The child was born with toxoplasmosis about three weeks before term.

The determination of acute acquired toxoplasmosis early in pregnancy is at present the only way known of avoiding the tragic cases of congenital toxoplasmosis.

When pregnant women complain of inexplicable tiredness or lymphadenopathy, with or without slight fever, the diagnosis toxoplasmosis should be considered and efforts made to establish the time of onset of the infection.

The exact extent of risk for the foetus becoming infected is not known yet Therefore, until greater experience is available, a termination of the pregnancy must be considered if clinical or serological manifestations of an acute toxoplasmosis are present in the mother.

2. The second group comprises women with antibodies when a new pregnancy commences. These women may previously have given birth to a child with congenital toxoplasmosis or have had a clinical acquired toxoplasmic infection.

Experience shows that women with one child with congenital toxoplasmosis can still complete a later pregnancy without risk for the new foetus becoming infected (*Sabin et al*, 1952, *Feldman* et al, 1956) This series includes 45 mothers with 56 healthy children and 204 pregnancies with no cases of congenital toxoplasmosis.

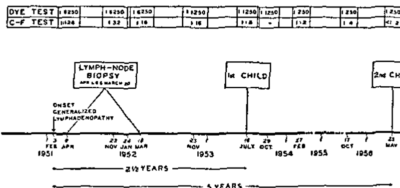


Fig. 7

Similarly, patients who have had acquired toxoplasmosis have later given birth to children free from toxoplasmosis (*Sim*, 1959). This is illustrated by Case No. 3 (Fig. 3) and by the case shown in Fig. 7.

SUMMARY AND CONCLUSIONS

1. The laboratory procedures for the routine diagnosis of human acquired toxoplasmosis are surveyed
2. Based upon observation of parasitologically verified cases of acquired toxoplasmosis with lymphadenopathy, a description is given of the clinical manifestations of its three forms – the febrile, the non-febrile and the sub-clinical.
3. Toxoplasma has been isolated from muscle and tonsillar tissue from patients with toxoplasmic lymphadenopathy
4. In toxoplasmosis acquisita lymphonodosa the modified Henle-Koch's postulates are fulfilled since (a) Toxoplasma has been isolated from enlarged lymph nodes removed from patients with characteristic clinical and serological findings, (b) the strains isolated have been identified as *Toxoplasma gondii* by morphological, serological, cultural and pathogenic criteria, (c) a significant rise in serological titres has been demonstrated in the acute phase of the disease simultaneous with the development of the symptoms and signs, when a comparison is made between acute and convalescent sera, (d) cases of a similar disease have been published as the result of laboratory infection.
5. The risk to the foetus caused by toxoplasmosis in pregnancy is discussed.

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State Bacteriological Laboratory, Stockholm, Sweden

ACQUIRED TOXOPLASMOSIS

GUNNEL HULDT

The laboratory diagnosis of toxoplasmosis has been carried out in Sweden since 1947, when the first case of congenital toxoplasmosis was observed there *(Magnusson)*.

As in other countries, it was mainly the congenital form which initially engaged our attention. The first Swedish cases of acquired toxoplasmosis to be reported (*Magnusson & Wahlgren, 1948*) were in clinically asymtomatic mothers of children with congenital toxoplasmosis.

However, in 1949 *Gard and Ljungstrom* found typically rising antibody titers against toxoplasmosis in a case of acute chorioretinitis in a 69-year-old man.

In 1950 *Gard & Magnusson* described a form of acquired toxoplasmosis associated with enlarged lymph nodes, chiefly cervical. In Denmark the same year, *Siim* observed and described an identical clinical picture. These authors reported a fairly well defined condition which greatly resembled infectious mononucleosis, except for the serological findings. – The Paul-Bunnell reaction is positive in mononucleosis, while toxoplasmosis is accompanied by an immunologic antibody response consisting of greatly elevated specific antibody titers.

In 1952 *Wising* reported a case of lymphadenopathy and acute chorioretinitis in a 31-year-old woman, who developed typically rising antibody titers against toxoplasmosis.

In 1952 *Siim* was able to isolate *Toxoplasma* organisms from involved lymph nodes in seven of eight cases of presumptive glandular toxoplasmosis with typical symptoms and high antibody titers. In so doing, he completed the chain of evidence demonstrating the etiology of these cases.

Glandular cases of similar type to those described by the Scandinavians have since been reported from other countries too, notably from Britain by *Skipper, Cathie,* and *Beverley and co-workers.*

In 1951 *Ström* described a case of laboratory infection with *Toxoplasma* in a young woman. She had an acute generalized infection associated with exanthema, pneumonitis, myocarditis and involvement of the central nervous system. She made a full recovery.

The cases of acquired toxoplasmosis diagnosed at our laboratory between

1952 and 1955 have been compiled. The diagnosis was established either on the basis of clinical data in conjunction with rising antibody titers and the histo-pathological findings or, in some cases, solely on the basis of titers which rose conspicuously or remained greatly elevated for more than three months (D.T. $\geq$ 1/1,000; C.F.T. $\geq$ 1/120). In each case at least three titrations were done over periods of not less than three months. Only in a few cases have biopsied lymph nodes been sent to the laboratory for isolation test, and all were negative. This might be due to the fact that they have reached the laboratory two or three days after biopsy. Since 1956 we try to inoculate mice within a few hours of biopsy and this has led to isolation of Toxoplasma in two cases of lymphadenopathy with high antibody titers against Toxoplasma.

Our complement fixation values may seem surprisingly high in comparison with those obtained at some other laboratories. The principal reason for this is that we express the titers as serum dilution in final dilution whereas many others express it as the initial dilution Converted to the initial dilution, our values will be about ¼ of those given here.

TABLE I

	Age in years					
	0-5	6-10	11-15	16-20	21-35	Over 35
Number of cases	0	3	0	2	21	2

Age distribution in 28 cases of acquired toxoplasmosis

The series includes a total of forty-seven cases – twenty-eight of them with clinical toxoplasmosis and nineteen with subclinical infection

Table I shows the age distribution in the clinical cases. The ages of the majority of these patients were from 21 to 35 years; two were 16 and 19 respectively. Of the two over 35, one was aged 49 and the other 69. The series includes three children of 6 to 7 years.

Worthy of note is that the great majority, or twenty-two of these twenty-eight, were females. This may possibly be largely due to the fact that interest in the diagnosis and subsequent observation of toxoplasmosis cases will naturally be greatest where female patients of fertile age are concerned

None of the clinical cases can be regarded as severe. Four patients had symptoms indicating a generalized infection After an initial period of about one week with marked tiredness, they developed moderate or high fever lasting for one or two weeks, accompanied by local symptoms from the respiratory or digestive tract. Early in the disease all the four patients showed relative lymphocytosis with some atypical white blood cells demonstrable both in the bone marrow and the peripheral blood. In the second week of the disease, lymphadenopathy was observed in three cases, in one case accom-

panied by hepato-splenomegalia and signs of myocardial lesion, in another case by encephalitis. Only in one case was a rash observed. All four cases recovered completely.

However, in the majority of the clinical cases the disease had a mild course. The patients had little or no fever. Several patients noticed enlarged lymph nodes *without any other signs of illness. Lymphadenopathy, which usually* persisted for a few months, was most frequently seen in the cervical region, submandibularly or close to the sternocleidomastoid muscle. In several cases enlargement of lymph nodes was observed also in the axillae and groins. In one case hilar lymphadenopathy was observed (Table II).

TABLE II

Total cases	Enlarged lymph nodes					Lymph node biopsy, suspected toxoplasmosis	Typical white blood picture		Fever	Enlarged spleen	Encephalitis	Exanthema
	Cervical	Axillary	Inguinal	Hilus pulm	Total with enlarged nodes		Number of cases investigated	Number of cases with pos findings				
25	21	10	4	1	24	12	19	12	7	4	1	1

Main clinical findings in 25 cases of acquired toxoplasmosis

Four patients had enlargement of the spleen. A white blood picture closely resembling that of infectious mononucleosis was observed in 12 of 19 cases *investigated.*

In some cases marked fatigue together with headache and mental depression followed the acute course of the disease. These symptoms lasted for several months and resembled those seen after viral meningo-encephalitis.

Of the three children, two were mild glandular cases. The third child, a 6-year-old girl, had attended an Out-Patient Department for abdominal pain of umbilical colic type. A routine blood test showed high titers, suggestive of toxoplasmosis (D.T. 1/1,250; C.F.T. 1/120). Further clinical investigation with respect to toxoplasmosis disclosed roentgenologic pulmonary lesions consisting of hilar enlargement and streaky densities running from the hili bilaterally, that is to say, of a type found in toxoplasmosis. These lesions persisted for about two months and produced no symptoms from the respiratory tract.

In the sub-clinical group, thirteen patients were mothers of newborn children with congenital toxoplasmosis. – Three of the remaining sub-clinical cases were detected at routine serological examinations: one at a Blood Donor Center, one at a Child Guidance Clinic (a 14-year-old girl), and one at a Gynecology Department. None of these patients had clinical symptoms.

The remaining three cases, which are classed as sub-clinical, are of diag-

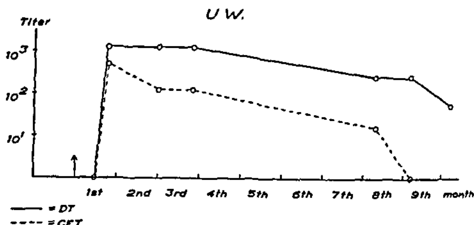
nostic interest. Two of them were children and one an adult Each of the two children – 6-year-old boys – had developed enlarged cervical lymph nodes. In both cases there were clinical grounds to suspect toxoplasmosis, and blood samples were sent to our laboratory. In each of them high antibody titers against toxoplasmosis persisted for several months. Histopathological examination of biopsied lymph nodes showed tuberculosis in one case (the diagnosis was verified by the finding of tubercle bacilli) and lymphatic leukemia in the other. In the third case, a 38-year-old woman had been hospitalized for fever and enlarged lymph nodes in the hili of the lungs Both the dye test and the complement fixation test were strongly positive (1/1,250 and 1/120 respectively). This case was initially regarded as toxoplasmosis until the patient, after a time, developed pleural exudation and roentgenologic pulmonary lesions typical of tuberculosis. This diagnosis was later verified bacteriologically.

In recent years the specificity of the dye test has been subjected to criticism by some authors *(Muhlpfordt, Awad)* who have claimed that antisera to other parasites such as sarcocystis and trichomonas may give cross reaction with toxoplasma. In the cases described here, both the dye test and the complement fixation test were strongly positive It seems reasonable, therefore, to assume that the patients concerned were infected with *Toxoplasma* organisms.

These cases are described as sub-clinical because in each of them some other cause of the relevant symptoms than toxoplasmosis was demonstrated. It is impossible, of course, to say if any of the general symptoms, such as fatigue, could have been partly due to toxoplasmosis. It is also difficult to judge whether toxoplasmosis could have developed coincidently with or before the disease in the affected lymph nodes. However, the biopsy specimens showed no histopathological signs of toxoplasmosis. – In my opinion, major practical importance is attached to the fact that in these cases the toxoplasmosis was not clinically manifest, despite the high titers Sub-clinical toxoplasmosis is known to be common, and hence it is not surprising to find high titers now and again, pointing to an infection of this kind in patients suffering from other diseases It is important to bear this in mind, so that if high antibody titers against toxoplasmosis coincide with some morbid condition, it will not necessarily be assumed that a relationship exists between the two

The immunological response to *Toxoplasma* infection can best be studied with the aid of a titer curve. Figure 1 shows the titers in a 30-year-old woman with typical, moderately pronounced, clinical toxoplasmosis in the form of lymphadenopathy and fever for about 10 days, followed by moderate headache and fatigue for a couple of months.

The onset is indicated by the arrow. About two weeks after the onset, the dye test and complement fixation test were negative, but 10 days later both of them were strongly positive. About six weeks later, the C F.T. had already



Dye test and complement fixation test in a case of acquired toxoplasmosis The arrow indicates the onset of clinical symptoms

Fig 1

commenced to fall. In a test taken nine months after the onset, the dye test titre had fallen also.

The mechanism of Toxoplasma infection in the human organism is still obscure. However, studies of the disease manifestations in man and in experimentally infected animals has provided some information. From the knowledge available it can be assumed that in the early weeks of the disease, before antibodies are demonstrable in the blood, there is probably a generalized infection where the parasites may be present for a time in the blood. It is likely that free parasites disappear from the blood stream coincidently with the appearance of humoral antibodies, although *Toxoplasma* organisms lodged within the cells may conceivably circulate in the blood and perhaps transmit the infection to the fetus even later. In the next stage of the disease, characterized by proliferative colonies in various organs, with tissue lesions and perhaps clinical organic symptoms, the antibody titers are high – indeed, at the beginning of this stage occasionally very high. The antibody production, it may be assumed, will continue to be stimulated as long as living parasites remain in the tissues. This means that in cases where terminal colonies or pseudocysts are formed, high titers will be demonstrable for a long period, sometimes several years, without the disease being active in the strict sense. – We can assume that not until the *Toxoplasma* organisms have died will the titers fall to negative or low values, even though in some cases, especially of congenital toxoplasmosis, toxoplasmin depots remaining in the tissues may stimulate the antibody production for an indefinite time thereafter.

From the series I have just reported, it is clear that acquired toxoplasmosis is usually a mild disease. We have had one fairly severe case, but this was

due to a laboratory accident where the infection dose may well have been massive. The patient in question made full recovery. Clinically, toxoplasmosis is important to bear in mind in the differential diagnosis of obscure glandular cases.

Although acquired toxoplasmosis is often an innocuous disease, it must be treated seriously as soon as it attacks women of fertile age, owing to the danger of transmission to the fetus in the event of pregnancy. Consequently, if toxoplasmosis is suspected clinically in women of this age, antibody tests should never be omitted. In the event of high titers, continuous checks should be made until the values have definitively fallen. Patients should be advised against pregnancy as long as the titers remain high.

SUMMARY

The series presented here comprises 46 cases of acquired toxoplasmosis, diagnosed serologically at the State Bacteriological Laboratory, Stockholm, during the years 1952–1955. Twenty-eight of them were clinical and 19 subclinical toxoplasmosis.

The clinical cases were all mild. Twenty-five had enlarged lymph nodes. Biopsies were taken in 12 cases and a histo-pathological pattern often seen in toxoplasmosis was observed. Isolation tests were performed in six cases, all of which were negative. Of the remaining three clinical cases, one had typical roentgenological lesions of the lungs, one had encephalitis, and the third fever and a typical blood picture.

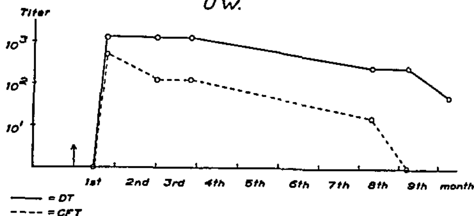
Three of the clinical cases were in children. Adults and children showed no difference in symptoms or severity of the disease

Of the sub-clinical cases, 13 were in mothers of newborn children with congenital toxoplasmosis. Three of the remaining sub-clinical cases had clinical symptoms – besides strongly positive titers – which, however, were due to other causes than toxoplasmosis (tuberculosis in two cases and lymphatic leukemia in one).

It is emphasized that clinical toxoplasmosis should not be diagnosed solely on the basis of high serological titers.

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Dye test and complement fixation test in a case of acquired toxoplasmosis The arrow indicates the onset of clinical symptoms.

Fig. 1

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Laboratory of Protozoology and Tropical Diseases,
and Hospital for Infectious Diseases, Faculty of Medicine,
University of Lyon, France

CONTRIBUTION TO THE STUDY OF ACQUIRED HUMAN TOXOPLASMOSIS

J. P. GARIN

Since our first work on toxoplasmosis, we have been particularly attracted by the acquired form of this parasitic infection, in which field it would seem that the more we learn, the greater is the scope

It is both a duty and a pleasure to recall that we owe much of our knowledge of certain of these acquired forms to Dr J. Chr. Siim, and much valuable information has been gained since he visited Lyon for the First International Congress on Infectious Pathology, organized by our chief, Professor P. Sédallian.

In my thesis published in 1953, I specified and classified the various clinical forms of acquired toxoplasmosis At least in the district of Lyon, two of these forms – the lymphadenopathic and the ocular – seem to occur very frequently.

Toxoplasmic lymphadenopathy is now recognizable by all, and there is no lack of proof of its existence. I propose today to give a report of three cases of toxoplasmic lymphadenopathy and sore throat, with isolation of the parasite from lymph node biopsies, in continuation of a primary work published together with Drs Sédallian and P Faure

On the other hand, toxoplasmic uveitis and chorioretinitis are much less well known. Therefore, we pay particular attention to these clinical forms, and review of the literature confirms their existence. I have chosen to report two cases out of the twelve observed together with Drs Paufique, Bonnamour and J. Rougier.

Case Histories

TOXOPLASMIC LYMPHADENOPATHY

CASE 1 (Fig 1) (Professor Dr. Croizat, No. 10,490)

Summary: Severe left sub-maxillary adenopathy with fever and lassitude in a 17-year-old girl Hypertrophy of the tonsils and spleen, monocytosis, negative Paul-Bunnell reaction, positive toxoplasma complement fixation and dye tests. Lymph node biopsy with isolation of the parasite by inocula-

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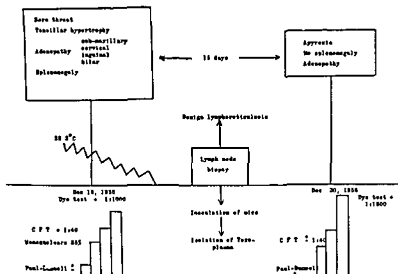


Fig 2

ocytes, mononuclear cells, some basophils, some large reticular cells, and a few polynuclear neutrophils Benign lymphoreticulosis.

Lymph node biopsy showed a lymphoreticulosis with some mitotic figures and slight vascular hyperplasia.

Some of the tests were negative Sedimentation rate, 5 mm per hour, Paul-Bunnell test negative; serological tests for typhoid, brucellosis, bacillary dysentery, Q-fever, ornithosis, psittacosis-lymphogranuloma, negative

Paper electrophoresis of the serum showed slight increase of alpha 2 and gamma globulin.

Ophthalmoscopy: negative.

The *clinical course* of the lymphadenopathy was of long duration The lassitude persisted for four months with periodic rises in temperature to 38° C, but both the lymph node and splenomegaly disappeared slowly.

Treatment consisted of oral administration of 1 g per day Vitamin C and 1 g calcium

Tests for toxoplasmosis:

Serology	18/2-55	18/3-55
Complement fixation	1 40	Negative
Sabin-Feldman test	1 500	1 600

Isolation of the parasite by inoculation into mice of lymph node suspension

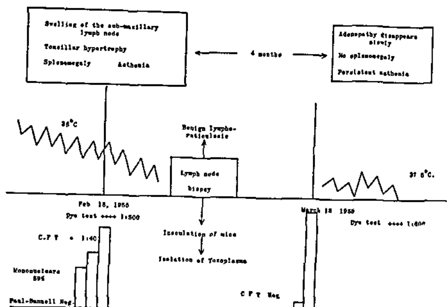


Fig. 1

tion into mice, confirmed by serological and pathological-anatomical examinations.

Case history· 17-year-old girl, examined for the first time on February 18th, 1955, on account of severe left-sided sub-maxillary adenopathy of about two weeks' duration, accompanied by temperature of 38° C and pronounced lassitude.

Physical examination revealed:

Left-sided sub-maxillary lymph node, nut-sized, mobile, not tender, slightly hard Bilateral adenopathy of the cervical lymph nodes, predominating on the left side. Hypertrophy of the tonsils.

Enlargement of the spleen, which was palpable a finger's breadth below the left curvature on inspiration and which could be seen on X-ray.

X-ray of the lungs showed right-sided hilar adenopathy, as the result of a primary tuberculous infection in 1949.

Tuberculin reaction now positive.

Laboratory examination showed:

Erythrocyte count 4,480,000. White blood cell count 8000. Haemoglobin 94 %. Colour index 1.04. *Differential counts:* Neutrophils 40 %, Eosinophils 1 %, Lymphocytes 15 %, Large lymphocytes 16 %, Monocytes 28 %.

Lymph node puncture showed a typical lymphoid hyperplasia with lymph-

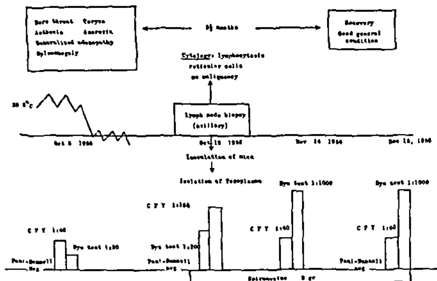


Fig. 3

antibodies with a S-F. titre of 1.100 could be demonstrated and typical histological lesions with intracellular toxoplasma and pseudocysts. On second passage there was evidence of a slight amount of peritoneal exudate.

The *clinical course* was uncomplicated. Ten days after the temperature had become normal, there was no sore throat, and the enlargement of the spleen had disappeared, though the adenopathy persisted.

Treatment was purely symptomatic.

CASE 3 (Fig. 3) (Professor Sédallian, No. 15,403).

Summary: Febrile rhinopharyngitis, poor general condition, and polyadenopathy in a 7-year-old boy Negative Paul-Bunnell reaction, toxoplasmic serology strongly positive, inoculation of lymph node suspension into mice positive

Case history. 7-year-old boy admitted to hospital on account of rhinopharyngitis with fever of several days' duration.

Previous history: The child has had measles, whooping cough, German measles and mumps. Four months previously vaccinated with B.C G.

Present complaint: Asthenia, anorexia, slight dysphagia of 8 days' duration Three days previous to admittance the temperature was 38.5° C; this became normal the day after hospitalization. At time of examination the voice was hoarse and there was a certain amount of discomfort on swallowing.

Appearance of antibodies in the mice after first passage detectable by S.-F. test 1:1000 25 days after inoculation. Despite the demonstration of toxoplasma on sub-inoculation, the animals did not die.

Pathological lesions in the inoculated animals: inflammatory perivascular granulomas and intracellular toxoplasma, particularly in the liver, myocardia and brain.

CASE 2 (Fig. 2) (Professor Sédallian, No. 14,544).

Summary Monocytic sore throat with fever, asthenia, hypertrophy of the sub-maxillary, cervical, inguineal and tracheo-bronchial lymph nodes in a 7-year-old boy. Enlargement of the spleen. Paul-Bunnell reaction positive. Complement fixation and S.-F. tests positive Isolation of the parasite by means of inoculation of lymph node biopsy into mice.

Case history: 7-year-old boy, examined for the first time on December 13th, 1955. Three days previously dysphagia had been observed, with lassitude, temperature of 38.3° C and sub-maxillary swelling.

Physical examination revealed:

Bilateral lymph nodes, not tender, no periadenitis, nut-sized, in the submaxillary, cervical and inguinal regions

Enanthema with hypertrophy of the tonsils, where a number of small whitish-ivory spots could be seen. Palate and uvula unaffected.

Enlargement of the spleen palpable a finger's breadth below the left curvature.

X-ray of the lungs showed voluminous nut-sized swelling of the hilar glands. Tuberculin test negative.

Laboratory examination showed.

Considerable monocytosis. Erythrocyte count 4,300,000. White cell count 13,000. Neutrophils 43 %, Eosinophils 2 %, Lymphocytes 12 %, Monocytes 43 %.

Lymph node biopsy showed diffuse lymphoreticulosis with slight sclerosis. Some reticular cells visible in the centre of the lymph nodes at the edge of the sinus showing mitotic figures. Other cells resembled epithelioid cells.

Serological tests showed:

	15/12-55	30/12-55
Paul-Bunnell reaction .	Slightly positive	Positive
S.-F. test	1:1000	1:1500
Complement fixation test .	1·40	1·40

Examination for toxoplasmosis:

A suspension of lymph nodes was inoculated into mice. Three weeks later

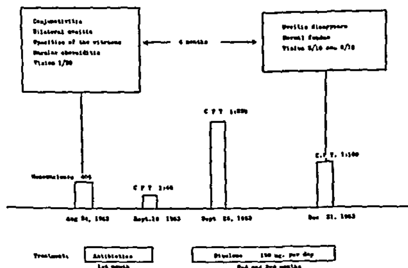


Fig 4

with diseased domestic animals, nor of food habits involving the consumption of raw meat. However, an elder sister had been admitted to hospital a short time previously on account of febrile sore throat. In her case the serological toxoplasmosis reactions were only slightly positive, the complement fixation reaction being 1.5 and the S.-F. reaction 1.20

Toxoplasmic Uveitis and Choroiditis

Case 4 (Fig. 4) (Professor Paufique). Summary of case reported in full by *Rougier & Garin.*

Summary: Severe bilateral uveitis, opacity of the vitreous and macular choroiditis in a 14-year-old girl. Positive toxoplasmosis complement fixation test with significant rise in antibody titre Cured by sulphone in the course of two months.

Case history· 14-year-old girl, who on August 24th, 1953, had a painful red right eye with impaired vision. Two days later the left eye was attacked

Physical examination revealed:

Satisfactory general condition, no lassitude, no fever, no sore throat, no adenopathy.

Right eye: vision 1/20. Anterior segment. conjunctival hyperaemia and pericorneal injection, aqueous flare, dust-like keratic precipitation, some ---erior synechiae.

Physical examination revealed:

Slightly sore throat, considerable serous nasal discharge, but no eruption.

Generalized lymphadenopathy, with a number of lymph nodes in the submaxillary, cervical, axillary, inguineal and popliteal regions.

X-ray of the chest showed a slight enlargement of the left hilus and enlargement of the spleen.

Laboratory examination showed:

Sedimentation rate 55 mm per hour.

Tuberculin reaction positive.

Smear of left axillary lymph node consisted mainly of normal lymphocytes and a large number of reticular cells, but there was no sign of malignancy.

Serological tests showed:

Paul-Bunnell reaction negative three consecutive times.

Strongly positive toxoplasmosis serological tests.

	Complement fixation						*S.-F. test*		
	1.5	1 20	1 40	1·80	1 160	1 320	1 20	1:120	1 1000
10/10	++++	+++	+++	not	made		+++++	0	0
17/10	+	+	++	++	+	0	+++++	++++	0
29/10	+++	++	++	not	made		+++++	++++	+
15/11	++++	+++	++	not	made		++++++	+++++	+++++
17/12	++++	+++	++	not	made		++++++	+++++	+++++

Toxoplasmosis test:

Left-sided axillary lymph node biopsy was carried out and a suspension of lymph node injected into 5 mice intraperitoneally. On December 13th, 1956, 4 S.-F. reactions positive, 1.1000. Suspension of brain, spleen and liver from these 4 mice was injected into 8 new mice. January 10th, 1957: 5 S.-F. positive reactions, 1 1000. December 21st, 1956: one mouse showed peritoneal exudate containing toxoplasma, at least four unquestionable parasites being seen. On December 26th, 1956, peritoneal exudate containing toxoplasma was present in 3 other mice, while the other 2 mice were negative. All inoculations made with the organs of these positive mice did not cause fatal infection in the mice.

The *course of the disease* was benign.

The child was *treated* with 2 g. Spiramycine per day for 2 months. On conclusion of the treatment the leucocytic picture showed lymphocytosis, the differential count being: neutrophils 34 %, eosinophils 3 %, lymphocytes 20 %, monocytes 43 %. The sedimentation rate had returned to 25 mm per hour. The child was able to return to school, the general condition being satisfactory.

From the *epidemiological* point of view, no evidence was found of contact

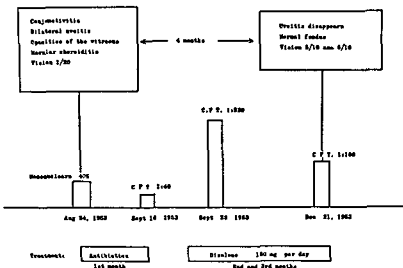


Fig 4

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Right eye. vision 1/20. Anterior segment. conjunctival hyperaemia and icorneal injection, aqueous flare, dust-like keratic precipitation, some terior synechiae.

Ophthalmoscopy: dense and dust-like opacities of the vitreous, macular choroiditis with stellate configuration round the macular lesion, oedematous retina, irregular tortuous vessels deep in the oedematous retina.

Left eye: Identical lesions in the anterior segment and fundus.

Laboratory examination showed:

Sedimentation rate 35 mm per hour.

Leucocytic count· 9000 with 40 mononuclear cells.

Lumbal puncture showed a clear liquid with hyperglycorrhachia of 1.15 g.

Serological tests for brucellosis, influenza and syphilis: negative.

Intradermal tuberculin test: negative.

Toxoplasmosis complement fixation reactions·

10/9-53	25/9-53	21/12-53
1.40	1.320	1·160

Clinical course and treatment:

During the first month treated with antibiotics (penicillin, streptomycin, aureomycin), local treatment with terramycin and cortisone Distinct improvement in the inflammatory condition of the anterior segment, but the vitreous remained opaque. In the fundus the papillary oedema and oedematous macular choroiditis remained in the left eye; in the right eye only the star-figure remained.

CASE 5 (Fig 5) (Professor Paufique). Summary of case reported in full by *Rougier & Garin.*

Summary: Impetigo of the eye lids in a 4-year-old boy Toxoplasmosis serology with high positive titres. Discovery of choroiditis with macular oedema in the right eye.

Case history. 4-year-old boy admitted to hospital March 18th, 1954, on account of an eruption on the cheek and eyelids.

Physical examination revealed.

Palpebral pyodermatitis with perinarinary lesions.

Conjunctival hyperaemia with slight sub-conjunctival haemorrhage.

Toxoplasmosis reaction positive.

Thorough examination carried out under general anaesthetic revealed: Right eye: phlycten-like corneolimbal lesion. In the fundus macular oedema with pseudo-macular hole Left eye normal.

The other examinations were negative, except for mucopurulent nasal discharge.

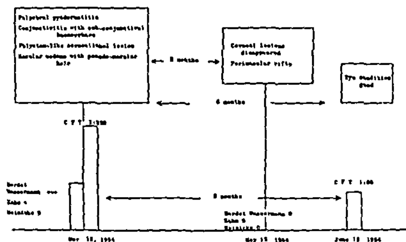


Fig 5

Laboratory examination showed

April 3: Bordet Wassermann +++, Kahn +, Meinicke 0
May 15. Bordet Wassermann 0, Kahn 0, Meinicke 0, Nelson test negative.
May 21 Lumbal puncture clear liquid, 2 cells, albumin 0 15, sugar 0 62, Bordet Wassermann 0.

All serological tests for typhoid, brucellosis, bacillary dysentery and rickettsiosis were negative.

Toxoplasmosis complement fixation test

7/4-54	12/6-54
1:320	1 80

Clinical course and treatment·

General treatment with penicillin and aureomycin, local treatment with aureomycin. The skin lesions disappeared in the course of some days Two months later the corneal lesions had disappeared. Some small perimacular rifts persisted in the fundus. Six months later the local conditions was excellent, there was no conjunctivitis and no lesions in the fundus.

DISCUSSION

These five cases call for some comments and remarks which can form the basis for evaluating the results of other works.

1. *Clinical remarks.*

A. *Toxoplasmic lymphadenopathy:* This condition may present different clinical aspects, from the simple febrile toxoplasmosis with generalized lymphadenopathy to forms with enanthema, enlargement of the spleen and even exanthema. Among the cases of toxoplasmic sore throat, all forms can be found: sore throat, sore throat with white spots, ulceromembranous and ulceronecrotic sore throat. However, as already indicated by Drs Sédallian and Faure, in addition attention should be paid to aphthous stomatitis and generalized aphthae, both being special clinical entities the etiology of which has up to now been somewhat mysterious. Finally, we have observed a new case where only the results of serology were positive while inoculations were negative. The common factor in all these forms lies in the clinical course, which is always of long duration, with persistent asthenia and adenopathy which is slow in disappearing

B. *Acquired ocular forms:* In my opinion, these are very interesting, since they are less well known. As I have shown in a critical review of the literature which I prepared with J. Rougier, the existence of these forms is well established. The best proof of this is reported by *Jacobs, Fair & Bickerton* They isolated toxoplasma from an enucleated eye from a case of chorioretinitis which had commenced eight years previously. From the clinical point of view, these forms should be isolated without repercussions on the general condition, or may be accompanied by slight general signs of toxoplasmic lymphadenopathy, including sore throat and meningitis. The ocular signs are different in the two cases of choroiditis I have mentioned, but they have certain points in common: (1) Involvement of the conjunctiva with severe and more or less purulent conjunctivitis, which perhaps represents the mode of entry. (2) Involvement of the uveal tract, as in our Case 3. This may be simply iritis with pericorneal injection, keratic precipitation, aqueous flare and posterior synechiae. The iritis may be torpid, recidivating or even complicated by hypopyon, or it may be posterior uveitis with dust-like opacities of the vitreous. (3) As regards choroiditis, this may be of several types. It may simply be an isolated area of choroiditis, or disseminating in foci in different stages of evolution, thus demonstrating former attacks. It may sometimes be simultaneous involvement of the retina (which is oedematous) or true chorioretinitis with deficiency of the field of vision. Among these forms of chorioretinitis, attention should be drawn to the possibility of Jensen's juxtapapillary chorioretinitis, with corresponding defects in the visual field.

The main characteristic in the course of an acquired attack of ocular toxoplasmosis is the combination of uveitis and an exudative focus of chorioretinitis when occurring in infants and young adults.

2. *Diagnostic remarks.*

The absolute proof in etiological diagnosis is the positive result of inoculation into animals. The tissues which give the most positive inoculations are lymph node and muscle biopsies. It is our experience that the strains isolated are slightly virulent for mice. They merely confirm the existence of a chronic infection, which can only be proved by means of serological and pathological examinations.

However, these parasitological tests are difficult to carry out in all cases, and thus it is necessary to be content with serological examinations. In our opinion, it is sufficient to examine by means of the complement fixation and Sabin-Feldman dye tests, thus gaining in the course of a few days evidence of significant variations in the antibody titres.

In our laboratory we use the following tests: (1) Complement fixation test with an antigen prepared from the peritoneal exudate of mice. The main precaution necessary is to wash the sediment repeatedly after centrifugation, in order to eliminate all traces of ascites which may be strongly anti-complementary. (2) The Sabin-Feldman dye test. This test is suitable for routine laboratory work and was taught to us by Dr Desmonts, Paris. Some precautions are necessary, i. e. to take the peritoneal exudate from the mice 48 hours after inoculation, and to store the accessory factor at — 40° C or on dry ice. The reading is simplified and accelerated by the use of phase contrast microscope.

Finally, apart from isolation of the parasite and specific sero-reactions, certain indirect tests may give rise to a suspicion of toxoplasmosis or indicate its biological signs. Lymphocytosis, whether or not associated with slight eosinophilia, when found in cases of sore throat would suggest infectious mononucleosis. It is therefore necessary to perform a Paul-Bunnell test, which in the majority of cases will be negative

However, as will be seen in our Case 2, this reaction may be positive. There is therefore the possibility of an association between toxoplasmosis and mononucleosis *Siim* has already acquainted us with the possible succession of these two conditions, mononucleosis may precede or follow toxoplasmic infection, or they may be present simultaneously.

In our Case 5, the serological tests for syphilis were temporarily positive. In this way this parasitic disease resembled a virus infection

3. *Epidemiological remarks.*

Here I should like to discuss the possible modes of infection in acquired toxoplasmosis. One mode of entry is certainly via the skin. Infection transmitted by ticks or by laboratory infection will not be discussed here.

A. There is evidence for and against the *conjunctival* mode of entry in the ocular forms. In favour is the observation of *Ansari & Minou* that fre-

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A. *Toxoplasmic lymphadenopathy:* This condition may present different clinical aspects, from the simple febrile toxoplasmosis with generalized lymphadenopathy to forms with enanthema, enlargement of the spleen and even exanthema. Among the cases of toxoplasmic sore throat, all forms can be found· sore throat, sore throat with white spots, ulceromembranous and ulceronecrotic sore throat. However, as already indicated by Drs Sédallian and Faure, in addition attention should be paid to aphthous stomatitis and generalized aphthae, both being special clinical entities the etiology of which has up to now been somewhat mysterious. Finally, we have observed a new case where only the results of serology were positive while inoculations were negative. The common factor in all these forms lies in the clinical course, which is always of long duration, with persistent asthenia and adenopathy which is slow in disappearing.

B. *Acquired ocular forms·* In my opinion, these are very interesting, since they are less well known. As I have shown in a critical review of the literature which I prepared with J. Rougier, the existence of these forms is well established. The best proof of this is reported by *Jacobs, Fair & Bickerton.* They isolated toxoplasma from an enucleated eye from a case of chorioretinitis which had commenced eight years previously. From the clinical point of view, these forms should be isolated without repercussions on the general condition, or may be accompanied by slight general signs of toxoplasmic lymphadenopathy, including sore throat and meningitis. The ocular signs are different in the two cases of choroiditis I have mentioned, but they have certain points in common (1) Involvement of the conjunctiva with severe and more or less purulent conjunctivitis, which perhaps represents the mode of entry (2) Involvement of the uveal tract, as in our Case 3. This may be simply iritis with pericorneal injection, keratic precipitation, aqueous flare and posterior synechiae. The iritis may be torpid, recidivating or even complicated by hypopyon, or it may be posterior uveitis with dust-like opacities of the vitreous. (3) As regards choroiditis, this may be of several types. It may simply be an isolated area of choroiditis, or disseminating in foci in different stages of evolution, thus demonstrating former attacks. It may sometimes be simultaneous involvement of the retina (which is oedematous) or true chorioretinitis with deficiency of the field of vision. Among these forms of chorioretinitis, attention should be drawn to the possibility of Jensen's juxtapapillary chorioretinitis, with corresponding defects in the visual field.

The main characteristic in the course of an acquired attack of ocular toxoplasmosis is the combination of uveitis and an exudative focus of chorioretinitis when occurring in infants and young adults.

Out of 75 sheep, 2 of the serological tests were positive
out of 23 goats, 2 of the serological tests were positive
out of 64 pigs, 1 of the serological tests was positive
out of 15 hens, 0 of the serological tests was positive

That is to say, out of 632 animals, 19 showed positive reactions, i e. about 3 per cent, with a high frequency among the goats. Meat for immediate consumption thus contained the parasite.

(b) It was demonstrated that in our district 8 per cent of the adults showed a positive complement fixation reaction Did the persons who eat underdone or badly cooked meat possess toxoplasmosis antibodies more frequently than the others?

We collected the serum of 11 persons with such eating habits, 6 of whom had tapeworm. All had eaten underdone beef and had become infected by that means. Three of the 5 persons, and 2 of the 6 with tapeworm, showed positive serological reactions.

6 patients with tapeworm		
	C.F.	S F
1	1.5	1 100
1	1·40	
	4 negative	

5 consumers of underdone meat		
	C F	S F
1	1 5	
1	0	1 10
1	0	1 10
	2 negative	

Thus, the number of persons in whom toxoplasmosis antibodies were found was more than four times higher in this group

The arguments for the digestive mode of infection in acquired toxoplasmosis are therefore quite reasonable

(c) Finally, as regards the mode of entry, it does not appear that toxoplasma multiply at the site of inoculation, which makes demonstration difficult in the skin, rhinopharynx, conjunctiva and the digestive membranes Following a phase of haematogenous dissemination, the toxoplasma multiply in the tissues which are rich in reticulo-endothelial cells, where they become encapsulated It is therefore not possible to determine the probable mode of entry in a given clinical condition. In effect, the presence of toxoplasma in the tonsils may be caused either by direct inoculation, or as a haematogenous infection

4. *Therapeutic remarks.*

Our cases do not permit any definite conclusions concerning the specific treatment of toxoplasmosis. As a matter of fact, in three of the cases recovery was accomplished by simple symptomatic treatment However, in Case 4, where the condition of the child appeared to be at a standstill, the institution of treatment with sulphone gave rapid and successful results. A dose of 150

TABLE I

Gastro-Intestinal Infection With Toxoplasmosis
Caused by Consumption of Raw Meat

Slaughterhouse animals		Positive sero-reactions	Controls	Consumers of raw meat: Cases with tapeworm (6)			Consumers of raw meat: Eaters of raw meat (5)		
					C.F.	S.F.		C.F.	S.F.
Cattle	450	14							
Calves	5	0	200	1	+ 1:5	+ 1.100	1	+ 1:5	
Sheep	75	2		1	+ 1:40		1	0	+ 1 10
Goats	23	2	persons	4 negative			1	0	+ 1.10
Swine	64	1					2 negative		
Hens	15	0	tested						
	632	19							
C.F + 1:10 S F. + 1.100 3 per cent			C.F. + 1.5 8 per cent				30 per cent		

quently the commencement of conjunctivitis is purulent. However, in our Case 5 conjunctival swab showed no toxoplasma, but examination was carried out after 15 days' treatment with collyrium. Against is the fact that involvement of the uveal tract and the choroid indicates an haematogenous infection.

B. We have already discussed the role of the *oral* mode of entry (Drs Sédallian and Faure). The main argument lies in the demonstration of the vegetative forms of toxoplasma from an ulcerated tonsil and from an aphthoid lingual swab In confirmation of this finding, *Sim* has shown that inoculation *of mice with tonsil biopsy may give positive results*

C. Finally, the *digestive* mode of entry is suggested. It is experimentally possible to find encapsulated forms in guinea-pigs and mice. These forms are particularly frequent in the muscles, and it is quite generally assumed that infection has occurred by means of badly cooked meat.

Proof of this means of infection has been studied as follows (Table I):

Firstly, by carrying out serological tests on the blood of animals at the Lyon slaughterhouse. Thanks are due to Dr Simintzis for granting facilities to Dr Coly who carried out the tests. Then we interrogated individuals on whom serological tests were carried out, as to whether they were in the habit of eating underdone or badly cooked meat.

(a) We carried out 632 serological tests on slaughterhouse animals, both by complement fixation 1:10 and the S -F. test 1:100, and the two combined. Out of 455 cattle examined, 14 were positive. It should be noted that the sero-reactions of 5 calves were all negative, which would seem to indicate that, as in the human species, the number of positive sero-reactions increases with age.

day, then 8 mg on the 21st, 25th and 28th days. Three mice died before the 16th day and 5 survived until the 72nd day. On this date four S.-F. tests on 5 animals were positive. It can thus be considered that the animals were still infected, though sub-inoculations were not carried out.

To conclude, Spiramycine is an antibiotic which is certainly active in experimental toxoplasmosis in mice, and can bring about recovery, provided that the dosage is high enough and the treatment period sufficiently long It is necessary to administer a dose of 300 to 400 mg./kg. per day for a minimum period of three weeks, followed by a lower dose for at least two weeks

This new antibiotic, the toxicity of which is practically nil, should take its place beside the other anti-toxoplasmosis remedies sulphapyrimidine, sulphone and pyrimethamine. There are possibly other uses for the drug, and it is to be hoped that some of the grave human diseases, even those which would formerly be considered fatal, will respond to it, if diagnosis is made at a sufficiently early stage.

The work of the past year has been rich in toxoplasmosis material. Study of the clinical aspects, diagnostic possibilities, epidemiologic considerations, and the efficacy of therapy, has provided us with more detailed information concerning this human parasitic disease, which has been so neglected in the past.

SUMMARY

Since preparing my thesis, in which I classified the different forms of human acquired toxoplasmosis, two forms have occurred very frequently in the Lyon district, viz. lymphadenopathy and uveitis-choroiditis.

A report is given of three cases of lymphadenopathy where toxoplasms were isolated, together with two cases of the ocular form, based solely on positive sero-reactions.

From the clinical point of view, aphthosis could be observed in the cases with lymphadenopathy

From the epidemiological point of view, the mode of entry would seem to be oral, via the tonsils and digestive system, and by ingestion of raw meat containing pseudocysts In this connection, we tested the serum of 632 animals from the slaughterhouse in Lyon, and found 19 positive, i e 3 per cent. In addition, the serum of 11 consumers of badly cooked meat was examined, and in three cases antibodies were found, i e. a frequency four times higher than in the whole group

From the therapeutic aspect, in addition to the sulphamide, sulphone and pyrimethamine drugs, all reported to be active in toxoplasmosis, a new antibiotic is introduced – Spiramycine – which is effective in the treatment of experimental toxoplasmosis in mice in a dosage of 400 mg./kg. per day,

mg. Disulone was administered daily for two months. This dose was well tolerated and recovery from the uveitis and choroiditis was expedited. The sharpness of vision increased from $^1/_{20}$ to $^5/_{10}$ in the right eye, and from $^1/_{30}$ to $^6/_{10}$ in the left eye. This activity, ascertained clinically, corresponded well to that found in the course of experimental toxoplasmosis in mice, in which we have observed the effect of treatment by examination of the liver.

Finally, I should like to report our experiments with a new French antibiotic called Spiramycine. The effect of this substance in toxoplasmosis has already been reported by *Bogacz*. Isolated from Streptomyces ambofaciens, it can be classified in the same group of drugs as erythromycine and carbomycine. Its toxicity for the mouse following subcutaneous challenge is: D.L. 50 % = 1.7 g./kg., i. e. 71 mg. for a mouse weighing 30 g.

We have used Spiramycine in a 1 per cent. solution of distilled water subcutaneously in one or two quotidian injections. These injections were well tolerated and absorbed by the mice. We have used mice with a weight of about 30 g.

A. An initial group of 10 mice inoculated with about 20,000 toxoplasms intraperitoneally were injected subcutaneously one hour after the inoculation with 2 mg. of the drug, and afterwards 4 mg. in two injections per day per mouse. Only one mouse was still alive after 10 days.

B. A second group was then inoculated subcutaneously with 20,000 toxoplasms. Two untreated mice died on the 8th day. Eight mice were treated, *receiving during the first 17 days two injections of 8 mg. of the drug, then* 4 mg every second day for 8 days, then 4 mg. once a week for a month. This therapy resulted in 7 of the 8 mice surviving for more than 8 months. On the 261st day the reaction of these 7 mice to the toxoplasmosis S.-F. test was negative and intraperitoneal inoculation of 500 toxoplasma caused their death after 7 days. It can thus be considered that they were sterile, otherwise they would not have been able to survive such a small reinoculation dose.

C. A third group of 8 mice, in which the controls died after 7 days, had been inoculated *intraperitoneally with 5000 toxoplasms and had received* 4 mg Spiramycine per day for 18 days. There were 6 survivors, but their condition was such that we administered a double dose (8 mg.) until the 32nd day. Despite this, four further mice died on the 19th, 24th and 60th days. However, 2 of the 8 mice survived for more than 7 months. On the 244th day the S.-F. test was negative and the inoculation carried out with suspensions of viscera, liver, spleen and brain were negative. It can thus be considered that the animals were sterile.

D. A fourth group were injected intraperitoneally with 10,000 toxoplasms. The two control mice died on the 7th day. Eight mice were treated subcutaneously with 4 mg. one hour after inoculation, 8 mg. in two quotidian injections for 7 days, 8 mg. as a single injection daily from the 8th to the 17th

Medical Policlinic, University of Würzburg, Germany

ON THE DIAGNOSIS AND CLINICAL ASPECTS OF ACQUIRED TOXOPLASMOSIS

H. Franke

In addition to the familiar aspects of *congenital* toxoplasmosis (*Bamatter, Frenkel* and *others*), increasing discussion has taken place during the last decade in almost all the countries concerned, regarding the clinical importance of *acquired* toxoplasmosis in both children and adults. This has been based mainly on the observation of cases where the infection was acquired in the laboratory or was confirmed by autopsy.

The methods developed by *Sabin* and co-workers for determining the presence of toxoplasma antibodies in serum have undoubtedly done a great deal towards increasing our knowledge of the *infection*, but not of the *disease itself*. Nevertheless, in the light of recent experience, it must be stressed most emphatically that not every case of toxoplasmosis of which we read, where diagnosis has been formed solely on the basis of serological tests without corresponding clinical findings, can stand up to closer investigation. Thus, we find in the literature only a strikingly small number of definite cases of manifest disease, as opposed to the large number of cases of latent infection. The question which to-day still occupies the attention of medical research, i. e. the accuracy of diagnosis of active acquired toxoplasmosis, depends – apart from the clinical findings and positive environmental history (diseased domestic or field animals) – on the continuously controlled results of the various tests which are applied. The various methods at our disposal for the diagnosis of toxoplasmosis (demonstration of the parasite, the inoculation of human material into animals, serological tests, the skin test, and pathological-histological reports on material removed in vivo) have varying significance in diagnosis. They should be applied with the greatest caution on account of the possibility of error.

The existence of acquired toxoplasmosis can be presumed with a probability converging on certainty when, as in 19 of our cases, the parasites are successfully demonstrated either (a) morphologically, located intracellularly, with the phase contrast microscope, (b) in smears from e g. the sediment of spinal fluid, or by biopsy (e. g diseased lymph nodes or particles of skin),

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latter in turn becomes negative sooner than the Sabin-Feldman test. As the progress of the two tests is different, both should be made frequently during the course of the illness, and the results evaluated carefully.

In two of our acute cases of freshly acquired toxoplasmosis with parasite test found positive both morphologically and by animal experiment, the dye test and complement fixation tests were at first negative but during the course of the illness became positive and increased in titre. On the other hand, the tests may also continue to be more or less positive some time after the clinical symptoms of the disease have disappeared. The diagnosis of manifest toxoplasmosis can be accepted as sufficiently probable when, in addition to the clinical symptoms, constantly controlled results of both tests rise to above eight times the normal and a very high titre (in extreme cases 1.36,000) is reached.

Specificity of the tests can hardly be doubted, when the titre in human blood increases to a very great extent, judging by material already published *(Frenkel, Mohr, Piekarski, Sabin, Siim)*. However, even a strongly positive Sabin-Feldman test cannot confirm whether it is a question of a primary acute to sub-chronic reactivated, or active secondary toxoplasmosis accompanied by another basic disease.

A solution of this important problem can only be reached by synoptic comparison of the clinical symptoms of the patient, including the personal and so-called environmental history, in conjunction with a series of serological tests undertaken at the same time.

From our experience with 60 cases, toxoplasmosis can only be said to be present with a certain degree of probability, when the serum shows a generally constant titre of 1.64 or above. In these cases the serological tests may only be evaluated in conjunction with the clinical picture and by attemps to demonstrate the parasite. Where the situation is not clear, the results of the serological tests must be considered with the utmost reserve. In many cases where the Sabin-Feldman test gives little result, e. g. indicating inactive toxoplasmosis accompanied by another basic disease, or inactive carriers of toxoplasma, we cannot speak of a manifest disease.

Clinical aspects of toxoplasmosis.

On the basis of the material from the literature (28 cases proved by the parasite test) and our own material (19 cases proved by the parasite test), together with 60 cases which can be considered as probable cases of toxoplasmosis from the clinical picture and serological tests, the following classification of toxoplasmosis in adults can be made: –

A. Congenital toxoplasmosis, first recognized as such in adult life.

or (c) by inoculation into animals which have first been proved to be free of toxoplasmosis. Only very rarely can the presence of terminal colonies of intracellularly-situated parasites be demonstrated, e. g. in the sediment of encephalomeningitis toxoplasmotica procured by animal experiment. These very sensitive terminal cysts can scarcely be confused with other formations in differential diagnosis. Extracellular toxoplasma in the smear must be separated from morphologically similar artefacts and fungi by means of special dyes (Gram, Feulgen and Berlin blue dyes).

In our last 10 cases of acute to sub-acute encephalomeningitis toxoplasmotica proved by demonstration of the parasite, we were able to presume, with the aid of the phase contrast microscope, the presence of extracellular toxoplasms in the cerebral fluid (sediment) at body temperature. These were recognizable not only by their shape (like segments of an orange) and inner structure (nucleus and volutin granules), but particularly by their peculiar rocking motion.

The diagnosis manifest toxoplasmosis is particularly certain when, as in one of our cases, in addition to the typical parasite test, the parasites are successfully transferred from the patient to young animals (mice or golden hamsters) ascertained beforehand to be free from toxoplasmosis. An isolated parasite may, however, only be classified as toxoplasma when the conditions laid down by *Sabin* are observed. Only rarely in suitable cases can the clinician make pathological-anatomical examination of glands (e. g. lymph glands *(Siim)* removed in vivo or fragments of organs) in order to verify his diagnosis.

Analagous with the investigations of *Siim*, we too succeeded in two cases of lymphadenitis toxoplasmotica in isolating the agents by lymph node biopsy, and in a further case in discovering the histologically recognizable terminal cysts in a skin nodule of a skin toxoplasmosis resembling erythema nodosum

However, as every pathologist stresses, a great deal of time is spent in finding what is after all a very limited number of toxoplasma in the autopsy material, and often the true form of the toxoplasma is lost during histological sections. Generally speaking, apart from the occasionally characteristic histological appearance *(Siim)*, we have to be guided by the pathognomonic terminal cysts.

If the parasite demonstration test is unsuccessful, in order to diagnose acquired toxoplasmosis the clinician must (apart from the clinical symptomatology which we shall describe later) rely on serological reactions to determine the presence of toxoplasmic antibodies. These are the Sabin-Feldman test and the complement fixation test *(Warren and Russ, Sabin)*.

Experiments on animals with human laboratory-acquired infections and with freshly-obtained toxoplasma from adults have shown that the dye test becomes positive sooner than the complement fixation test, and that the

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A. Congenital toxoplasmosis, first recognized as such in adult life

B. Acquired toxoplasmosis, with its various types of development according to *Frenkel* (acute stage I, sub-acute stage II, chronic stage III, latent forms, and "burnt-out" forms.

I. Primary toxoplasmosis with:

1) polysymptomatic clinical appearance
   (a) acute, feverish exanthematous form
   (b) central nervous form (encephalitis, encephalomyelitis, meningitis)
   (c) predominantly pulmonary form (interstitial pneumonia)
   (d) predominantly enteric form (enterocolitis haemorrhagica)
2) oligosymptomatic clinical appearance with involvement of certain organs
   (a) lymphadenitis toxoplasmotica
   (b) eye affections (chorioditis toxoplasmotica, etc.)
   (c) papular skin diseases (erythema nodosum)
   (d) cardiac form (myocarditis)
   (e) isolated hepatitis, lienitis, myositis, orchitis, etc.

II. Reactivated toxoplasmosis, syndromes as in I,1 and I,2.

III Active toxoplasmosis (secondary toxoplasmosis, toxoplasmosis accompanied by another active basic disease, e. g. toxoplasmosis and paratyphus), syndromes as in I,1 and I,2.

IV. Inactive toxoplasmosis accompanied by another basic disease, e. g. multiple sclerosis or thrombosis obliterans.

V. Carriers of toxoplasma without toxoplasmosis.

Apart from the type of congenital toxoplasmosis which is not recognized until later in adult life, we must distinguish basically between primary acquired toxoplasmosis in its various forms of development (acute, sub-acute, chronic, latent, burnt-out) on the one hand, and on the other reactivated and secondary or accompanying toxoplasmosis, either active or inactive.

The existence of acquired toxoplasmosis is clearly demonstrated by the published reports of laboratory infections with toxoplasma parasites (*Bengtsson, Franceschetti, Bamatter, Mohr et al., Giroud et al., Sabin, Ström, Magnusson, Beverley et al.)* This is also confirmed by the fact that, in addition to the clinical findings, in more than half the cases observed by us, domestic or field animals with toxoplasmosis, with which the patient has been in close contact, can be assumed to be the transmitters of the disease.

In three of our cases of primary toxoplasmosis proved by demonstration of the parasite, an acute feverish exanthematous condition developed, clearly as the result of generalization, resembling in appearance typhus or "Rocky Mountain fever", with slight spleen tumor and bronchitis.

In the course of the disease, more or less clear symptoms of encephalomeningitis appeared in every case. We observed 11 cases, proved by demon-

stration of the parasite, and 30 cases diagnosed with a degree of probability on the basis of the clinical appearance and serological tests which had risen to a pathological extent. In all these cases the central nervous form of acquired toxoplasmosis was present.

The clinical features in patients developing acute to chronic encephalomyelomeningitis may be relatively characteristic (*Schwarz, Kirk, Rose* and *Fry*) as in two of our cases, with both general symptoms (severe headache with giddiness and depression, increasing sometimes to cerebral coma) and localized neurological symptoms dependent on the site of the toxoplasmic encephalitis. My colleagues *Horst* and *Hann* drew attention to instructive encephalographic findings in these patients. One patient died and post mortem showed encephalitis toxoplasmotica.

In the majority of our cases, the cerebral meninges showed meningeal inflammation with pleocytosis, normomastic curve of the meningitis type, and sometimes parasites demonstrable in the cerebral fluid

In atypical cases of encephalitis toxoplasmotica, recognition of the central nervous form of toxoplasmosis can be extremely difficult if the parasite test fails, even taking into account the neurological symptomatology of the differential diagnoses.

The case which we considered to be a pulmonary form of acquired toxoplasmosis had, if we exclude a virus (e.g. Queensland fever), the clinical and radiological appearence of an interstitial pneumonia which commenced like influenza. At the same time swelling of the lymph glands, remarkable eosinophilia of the blood followed by monocytosis, and highly positive reaction to the Sabin-Feldman and complement fixation tests, were observed. After a further four weeks this patient developed encephalomeningitis proved by demonstration of the parasite, clearly on account of dispersion via the blood.

In five of our cases we presumed a probably enterocolitic development. Apart from the intestinal symptoms and the steadily increasing titres in the serological tests, what were later to become complications typical for toxoplasmosis (i. e. encephalomeningitis, swelling of glands, and peribronchitis) developed.

In the case of acute toxoplasmosis with polysymptomatic clinical appearance, various combinations of the symptoms described above can appear regularly in the same patient.

On the basis of the published material and our own experience, acquired toxoplasmosis can also develop primarily oligosymptomatically with occasional involvement of the lymph glands (*Siim*), the heart (*Bengtsson, Mohr*), eyes (*Straub* and *Otto*), skin (*Franke* and *Schuermann*), liver (*Callahen*) and spleen (*Bamatter*).

Apart from the method of dispersion, the symptomatology of these cases depends presumably on the number of parasites in the organ concerned.

B. Acquired toxoplasmosis, with its various types of development according to *Frenkel* (acute stage I, sub-acute stage II, chronic stage III, latent forms, and "burnt-out" forms.

I. Primary toxoplasmosis with:

1) polysymptomatic clinical appearance
   (a) acute, feverish exanthematous form
   (b) central nervous form (encephalitis, encephalomyelitis, meningitis)
   (c) predominantly pulmonary form (interstitial pneumonia)
   (d) predominantly enteric form (enterocolitis haemorrhagica)
2) oligosymptomatic clinical appearance with involvement of certain organs
   (a) lymphadenitis toxoplasmotica
   (b) eye affections (chorioditis toxoplasmotica, etc.)
   (c) papular skin diseases (erythema nodosum)
   (d) cardiac form (myocarditis)
   (e) isolated hepatitis, lienitis, myositis, orchitis, etc.

II. Reactivated toxoplasmosis, syndromes as in I,1 and I,2.

III. Active toxoplasmosis (secondary toxoplasmosis, toxoplasmosis accompanied by another active basic disease, e g toxoplasmosis and paratyphus), syndromes as in I,1 and I,2.

IV. Inactive toxoplasmosis accompanied by another basic disease, e. g. multiple sclerosis or thrombosis obliterans.

V. Carriers of toxoplasma without toxoplasmosis.

Apart from the type of congenital toxoplasmosis which is not recognized until later in adult life, we must distinguish basically between primary acquired toxoplasmosis in its various forms of development (acute, sub-acute, chronic, latent, burnt-out) on the one hand, and on the other reactivated and secondary or accompanying toxoplasmosis, either active or inactive

The existence of acquired toxoplasmosis is clearly demonstrated by the published reports of laboratory infections with toxoplasma parasites *(Bengtsson, Franceschetti, Bamatter, Mohr et al., Giroud et al , Sabin, Strom, Magnusson, Beverley et al.).* This is also confirmed by the fact that, in addition to the clinical findings, in more than half the cases observed by us, domestic or field animals with toxoplasmosis, with which the patient has been in close contact, can be assumed to be the transmitters of the disease.

In three of our cases of primary toxoplasmosis proved by demonstration of the parasite, an acute feverish exanthematous condition developed, clearly as the result of generalization, resembling in appearance typhus or "Rocky Mountain fever", with slight spleen tumor and bronchitis.

In the course of the disease, more or less clear symptoms of encephalomeningitis appeared in every case. We observed 11 cases, proved by demon-

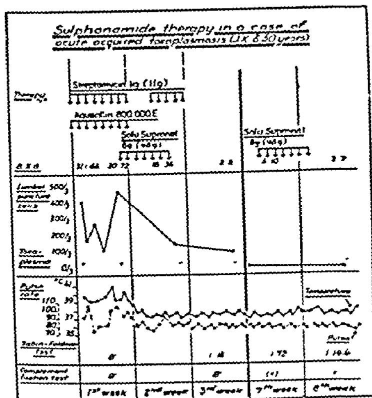


Fig. 2

Reactivated toxoplasmosis

It is our experience that cases of human toxoplasmosis, especially in adults, developing to an acute stage have not always commenced as primarily acute forms. Carefully collected data from case histories indicates that generally in such cases the first symptoms, and sometimes also signs of toxoplasmosis have been noted years before, and that certain factors tending to reduce the general resistance, e. g. pregnancy, physical overstrain or subjection to exceptional temperature changes, are capable of activating the previously latent toxoplasmosis. We have ourselves observed 12 such cases of reactivated toxoplasmosis.

Active accompanying toxoplasmosis.

The clinician will always be able to diagnose so called active accompanying toxoplasmosis in conjunction with another basic illness when the patient's symptoms can only be explained by the interaction of both affections. Of the 12 cases of active accompanying toxoplasmosis which we have observed,

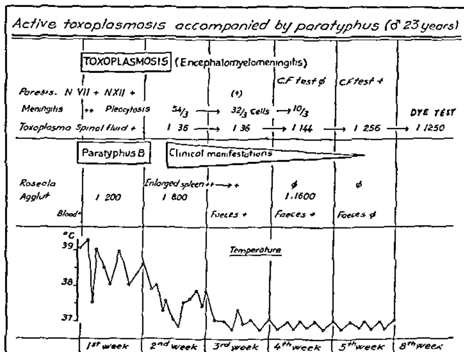


Fig 1

Analogous with the investigations of *Siim*, we are able in two cases of the oligosymptomatic form of lymphadenitis toxoplasmotica with only slight Paul-Bunnell reaction, to discover the parasite in the punctate of an enlarged cervical lymph gland. In our case the blood test gave only a moderate monocytosis of 12 to 14 per cent

In six of our cases of acute toxoplasmosis with encephalomeningitis proved by demonstration of the parasite, we observed during the progress of the disease fresh chorioretinal foci with sub-retinal exudation, particularly in the macular region of the retina.

Cases of oligosymptomatic toxoplasmosis of the skin in the late stages are rare both in the literature and in our own experience. In one of our cases an *isolated skin toxoplasmosis, resembling a coarse granular erythema nodosum*, developed many months after the disappearance of the acute encephalomeningitis toxoplasmotica. Parasites were found in the skin node punctate and a pathognomonic terminal cyst in the histological section from the lymph node biopsy.

Oligosymptomatic cases with predominant involvement of the heart *(Bengtsson, Mohr)*, liver, i.e. hepatitis toxoplasmotica (18 fatal cases by *Callahan*), muscular system, i.e. myositis (e.g. myositis of the gastrocnemius muscles), and the kidneys *(Kean* and *Grocott)* have not been observed by us. They are mentioned here merely for the sake of completeness.

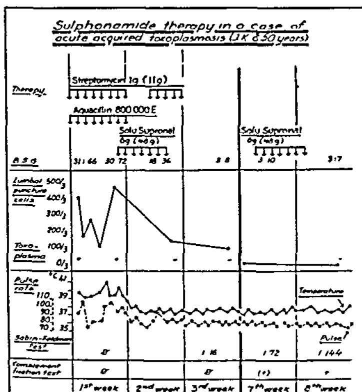


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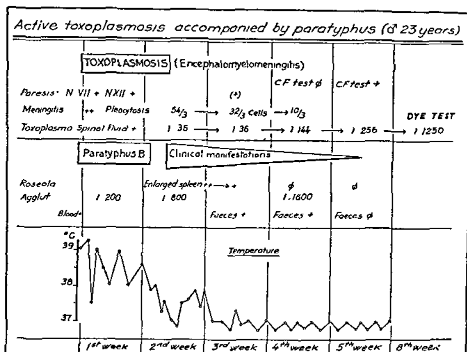


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we shall illustrate by diagrams the interaction of active toxoplasmosis proved by demonstration of the parasite, accompanied by paratyphoid fever (Fig 1)

Lack of space precludes discussion of the inactive forms of acquired toxoplasmosis which we have observed, i. e. inactive toxoplasmosis accompanied by another basic disease, and latent carriers of toxoplasma without toxoplasmosis. The latter forms are extremely problematical and can be approached only with the greatest caution

Our aim must be *the early diagnosis of acute toxoplasmosis*, as our experience has shown that these can best be treated intravenously with sulphonamides followed by Daraprim treatment (Fig 2)

The treatment of the sub-chronic to chronic forms of acquired toxoplasmosis is even to-day still far from satisfactory, despite its clinical importance, e. g. in the prevention of infection of the foetus in the case of pregnant women with latent toxoplasmosis.

SUMMARY

The problem of diagnosis and the clinical aspects of toxoplasmosis in adults are discussed on the basis of the observation of 79 cases of toxoplasmosis, 19 proved by demonstration of the parasite, and 60 diagnosed as probable from the clinical findings and serological tests

Mention is made of the various tests performed to assist diagnosis (parasite test, animal experiments, serological and skin tests, pathological-histological examination of biopsy material).

Only the difficult parasite test is suitable for early diagnosis, as serological tests become positive only several weeks subsequent to infection.

When the parasite test fails, diagnosis of acquired toxoplasmosis can only be made with any degree of probability on the basis of more or less pathognomonic symptomatology in conjunction with detailed study of environmental history (diseased domestic animals, etc) and constantly rising titres in serological tests (Sabin-Feldman and complement fixation tests).

Apart from congenital toxoplasmosis, distinction must be made between primary acquired toxoplasmosis with its various forms of development (acute, sub-acute, chronic, latent, burnt-out) and the reactivated form and secondary or accompanying form, either active or inactive.

The polysymptomatic and *oligosymptomatic aspects of the various forms* of acquired toxoplasmosis are discussed briefly on the basis of cases observed, and reference is made to the problem of treatment.

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Clinique de Puériculture, Hopital St. Vincent de Paul,
Paris, France

OBSERVATIONS ON BIOLOGICAL AND CLINICAL DIAGNOSIS OF ACQUIRED TOXOPLASMOSIS IN CHILDREN

G. Desmonts

We have been studying Toxoplasmosis since 1947 under the supervision of Professor Marcel Lelong. Here are some of our observations concerning the biological diagnosis, and the first results obtained in the acquired form of the disease in children.

Serological Tests.

Each serum is tested by two methods. – The Sabin-Feldman dye test (1) and the complement fixation test.

A. *Sabin-Feldman dye test.* It was very difficult for us to carry out this test as a routine. For five years the results were very irregular, but our confidence in the authors kept us from giving up. Since 1953 we have overcome the difficulties and the results are now satisfying.

Some of the details of our method are different from those of Sabin-Feldman (2): –

Different time of incubation (37° C) depending on whether the controls are more or less quickly significant (usually between 10 and 30 minutes).

Reading with a phase contrast microscope without previous staining (2, 3, 4). The results are satisfactory when negative controls show less than 10 % lysed parasites (which would be unstained) and positive controls over 90 %. Such results are obtained regularly when the freshly taken exudate shows many cells full of parasites, and only a few located extracellularly. If there are too many extracellular parasites, the difference between negative and [illegible]

[illegible] of infected mice (cells and parasites centrifuged and washed). Control antigen is made from spleen of healthy mice, according to *Frenkel's* method for toxoplasmin preparation (6). The antigen-antibody complement mixtures are incubated for 16 to 20 hours at + 4° C. The haemolytic system is then titrated according to the Demanche method.

TABLE I

Results of 1814 sera tested in 1954 and 1955

Sabin-Feldman titre	Titre of complement-fixing antibodies											No of sera
	Neg	Undil.	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	
1:10,000								1	6	3	2	12
1:1000		1	2	8	19	30	46	54	26	3	1	190
1:100	6	57	88	124	108	37	19	4				443
1:10	144	178	62	24	5							413
Neg	743	13										756
										Total		1814

Correlation between the two serological tests.

The table shows that the correlation between the two tests is fairly good However, we obtained 13 slightly positive C F tests (undiluted serum) in sera in which no dye test antibody could be demonstrated. These are probably non-specific reactions 150 Sabin-Feldman tests are slightly positive (1:10 and 1.100), though the C.F tests are negative. Thus, the latter test is not as certain as the Sabin-Feldman test, and it is well known that the complement-fixing antibodies disappear sooner after infection Finally, some of the highly positive Sabin-Feldman tests are associated with a low rate of complement-fixing antibodies This signifies often, *though not always*, a recent infection with rising titre of C F. antibodies.

Parasitological investigation

Inoculations are made into albino mice.

The main points in our method are (7) –

(a) Each animal is tested before inoculation (Sabin-Feldman test)

(b) Further serological tests are performed during subsequent weeks. If after 45 days the serological tests are still negative, the inoculation should be considered as negative, experience has shown that it is useless to carry out sub-passages. However, if the serological tests become positive (usually between the 20th and 30th day), brain tissue autopsy examination shows cysts, and the injection of the brain tissue suspension into clean white mice treated with cortisone produces more virulent infection

Relation between serological tests and inoculation.

1 Inoculation of brain tissue of children with congenital Toxoplasmosis is most often positive.

The high percentage of positive results in congenital Toxoplasmosis shown in the table confirms our confidence in the reliability of the serological tests.

TABLE II

Brain tissue inoculation

Name	Age	Antibody titres[1]		No of mice[2]	Remarks
		S.-F	C F.		
SE.	36 hrs	?	?	0/6	*Tests on mother* S.-F. 1.8000, C.F. 1.128. Brain tissue was unfortunately frozen before inoculation
PE.	16 days	?	?	8/8	*Tests on mother*: S.-F. 1·1600, C.F. not performed.
DO	50 days	1.1000	1 10	2/2	(a) The child was treated with pyrimethamine and sulfonamids from the age of one week old. (b) Blood inoculation on the 5th day was positive.
DU	*53 days*	*?*	*1 64*	*12/12*	
CO.	6 mths.	1 2000	1·160	4/4	Myocardium inoculation was also positive.
PO.	12 mths.	1 500	1 32	6/16	Positive inoculation was also obtained at five months from muscle biopsy.
VI.	15 mths	1 1000	1.64	1/2	Brain tissue was obtained by cortical biopsy
PI	19 mths.	1.2000	1·100	0/4	Brain tissue was obtained by cortical biopsy.
VA.	35 mths.	1 800	1.8	1/6	Toxoplasma cysts were also seen in brain sections.
					Totals: 9 patients 7 positive results

1. The antibody titre given is the last one obtained before the death of the child
2. Denominator: number of mice inoculated.
   Numerator. number of mice infected

Thus we have several times inoculated the brain tissue of newborn children without toxoplasma antibodies and have never obtained positive inoculation.

2. To confirm the etiological significance of toxoplasma in lymph node diseases, we have injected lymph nodes taken from 57 patients into mice. These patients can be divided into two groups: –

Group A (controls): This includes 30 inoculations with lymph nodes taken from any patients during surgical operations. At the same time serological tests were performed, with the following results: –

Negative 18
Slightly positive 10
(Sabin-Feldman tests between 1:10 and 1:200, C.F.tests between 0 and 1 4)
Highly positive 2
(Sabin-Feldman test 1:1000, C F. test 1 32)

All the inoculations were negative

Group B· Lymph node biopsy was requested from the 27 other patients, since their serological titres and clinical features gave rise to the suspicion of toxoplasmic adenopathy. The results are shown in Table III.

TABLE III

Results of 27 lymph node biopsies

Sabin-Feldman test	Compl fixation test	Inoculation
1 250	1 32	0
1 500	1 8	0
1.500	1 10	0
1 500	1 10	0
1.500	1 20	0
1:500	1 64	0
?	1 16	0
1 1000	1 16	0
1 1000	1 16	0
1:1000	1 16	0
1 1000	1 64	Positive
1:1250	1·32	0
1.2000	1:10	Positive

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1:1000	1 16	0
1:1000	1 64	Positive
1 1250	1·32	0
1 2000	1 10	Positive
1·2000	1·16	0
1·2000	1.50	Positive
1 2500	1 50	0
1 2500	1 50	Positive
1:2500	1 64	Positive
1 2500	1 100	0
1 4000	1 64	Positive
1 4000	1 128	Positive
1.4000	1 200	0
1 4000	1 200	Positive
1 6250	1.32	0
1 8000	1 64	Positive
1 10,000	1 200	0
1 16,000	1·128	Positive
Totals 10 positive inoculations out of 27		

Only once was isolation successful when the dye test antibody titre was less than 1 2000. However, 9 out of 15 inoculations confirm the diagnosis

when the antibody titre is more than or equal to 1:2000. It can thus be considered that such antibody titres are significant.

Clinical symptomatology.

It is now a common occurrence for us to find highly positive Toxoplasmosis tests when patients with a clinical syndrome suggesting infectious mononucleosis have a negative heterophile agglutination test. However, a positive reaction does not justify the diagnosis of Toxoplasmosis, owing to the fact that it so frequently occurs in normal subjects. Therefore, in order to obtain an impression of the clinical picture, we selected 16 cases, as follows. –

2 confirmed by increasing antibody titre and positive inoculation,
4 confirmed by a very high antibody titre and positive inoculation,
10 confirmed by a definitely increasing antibody titre, with a simultaneous evolution of the clinical syndrome, but where biopsy could not be performed [1]

The main signs we observed are: polyadenopathy, fever and asthenia, skin rash, splenomegaly, blood count changes. One case was complicated with encephalitis.

Adenopathy Swelling of lymph nodes is the most constant sign. In 13 cases adenopathy was the reason for the medical examination, or was the main result of the clinical examination Though sometimes said to be tender, the lymph nodes are generally painless, or only tender on palpation. They are of average size (hazel-nut or almond sized), firm though not hard, without any periadenitis They may be found in all areas, though mostly in the cervical, axillary, inguinal regions, and more specifically in the occipital and trapezian areas, appearing one after the other within a few days. They last for quite a long time – at least several weeks – and may remain easily palpable for several months.

The lymph node manifestations may be more slight (3 cases). apparently common polymicroadenopathy, or even lymph node swelling confined to one region, discovered only on systematic examination.

Fever and asthenia. Fever is a frequent, but not constant, sign (10 cases out of 16) The fever might be high (39° to 40°) for a few days, followed by a low fever for several weeks, or more often a low feverish condition only, which may last for several weeks and sometimes for even one or two months. The fever is accompanied by marked asthenia. Asthenia can also be found in apyretic patients

1. Since the present article was written, the number of cases observed has increased to nearly 100, but no great change in the conclusions drawn from our first experiences has been found.

Skin rash: Rash seems to be an infrequent sign. However, in 3 cases out of 16 the disease started with a morbiliform rash, in one case with purpuric elements.

Splenomegaly Splenomegaly is seldom found (2 out of 16 cases), and in these two cases it was only slight

Blood count changes These are very frequent (11 out of 16 cases). Generally, the number of leucocytes is almost normal or slightly increased but with a reduced proportion of polymorphonuclear cells. The main observation is the presence of hyperbasophilic mononuclear cells, comparable to those of infectious mononucleosis. Sometimes eosinophilia may appear (5 times 7 %, once 16 %, once 43 %)

The course of the disease has always proceeded towards spontaneous recovery without complications, except in one case with clinically curable encephalitis.

To sum up, our 16 cases have been as follows. –

- One eruptive fever with encephalitis,
- Three unaccounted febrile conditions with glandular enlargement, discovered only on routine check-up,
- Eleven with polyadenopathy, with or without fever, resembling a mild form of infectious mononucleosis;
- One almost asymptomatic form – a child in excellent general condition whose serological test was highly positive. The child had only an apparently common polymicroadenopathy This case forms the link with the inapparent form of the infection.

DISCUSSION

Our experience entirely confirms *Sim's* work (8) and his description of the clinical picture of toxoplasmic adenopathy This seems to be a frequent disease during childhood. Diagnosis on clinical grounds alone is never justified but should be confirmed by biological tests. The most convincing proof is isolation of the parasite. However, biopsy may not be easily obtained from patients with such a mild form of the disease. Fortunately, one can rely on the serological tests with great confidence

Some authors (9) speak of the non-specificity of the dye test Our purpose here is not to discuss their experimental works, but our results and the good concordance between serological tests and inoculation seem to us to establish the reliability of serological tests in human beings.

High titres of antibodies (Sabin-Feldman test over 1.2000, C.F. test over 1 50) are probably significant in most cases, but it is better if a rising titre is observed. At least two specimens are necessary – the first as soon as possible, and the second about three weeks later. Both sera must be examined in the same series of tests in order to avoid any difference due to technique

when the antibody titre is more than or equal to 1:2000. It can thus be considered that such antibody titres are significant

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10 *confirmed by a definitely increasing antibody titre, with a simultaneous* evolution of the clinical syndrome, but where biopsy could not be performed.[1]

The main signs we observed are: polyadenopathy, fever and asthenia, skin rash, splenomegaly, blood count changes. One case was complicated with encephalitis.

Adenopathy. Swelling of lymph nodes is the most constant sign. In 13 cases adenopathy was the reason for the medical examination, or was the main result of the clinical examination Though sometimes said to be tender, the lymph nodes are generally painless, or only tender on palpation. They are of average size (hazel-nut or almond sized), firm though not hard, without any periadenitis. They may be found in all areas, though mostly in the cervical, *axillary, inguinal regions, and more specifically in the occipital and trapezian* areas, appearing one after the other within a few days. They last for quite a long time – at least several weeks – and may remain easily palpable for several months.

The lymph node manifestations may be more slight (3 cases) apparently common polymicroadenopathy, or even lymph node swelling confined to one region, discovered only on systematic examination.

Fever and asthenia· Fever is a frequent, but not constant, sign (10 cases out of 16). The fever might be high (39° to 40°) for a few days, followed by a low fever for several weeks, or more often a low feverish condition only, which may last for several weeks and sometimes for even one or two months The fever is accompanied by marked asthenia. Asthenia can also be found in apyretic patients.

1 Since the present article was written, the number of cases observed has increased to nearly 100, but no great change in the conclusions drawn from our first experiences has been found

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The observations are either a parallel increase in titre in both tests, or a rising titre of C.F. antibodies alone associated with an already high Sabin-Feldman test.

It is difficult to determine with accuracy the date of the appearance of antibodies, just as it is often impossible to recognize the insidious beginning of the disease. We believe, however, that the dye test antibodies appear during the second or third week of the disease, *while the C.F. test becomes positive* during the third or fourth week.

However, one must be careful about the non-specificity of increasing antibody titres. Without having any accurate proof, we have the feeling that some infections are able to increase the tests temporarily (especially the Sabin-Feldman test) Some highly positive Sabin-Feldman tests associated with low C.F. tests are not due to an evoluting Toxoplasmosis, but rather to a non-specific reactivation of previous antibodies by some virus disease.

It is also of importance for the significance of the tests to know the rate of decrease of the titres. In fact, they seem to decrease slowly. In children with congenital Toxoplasmosis, the titres generally remain high for several years; the results published in *Couvreur's* thesis are very close to *Feldman's* (10).

As regards acquired Toxoplasmosis, our follow-up material is insufficient, but we have observed important individual differences. The titres of some children fall from Sabin-Feldman 1.5000 to Sabin-Feldman 1:10 within 18 months, while others remain at a very high level (e.g. 1.2500) for two years.

The decrease in antibody titres during the years following infection explains the results of serological surveys in the normal population, viz. that most subjects are infected during childhood. Therefore, while the number of positive reactors is greater in older groups, high titres are more frequent in younger groups (10, 11).

Obstetricians should bear in mind the clinical signs of the infection. *Couvreur* (12) *has shown in his thesis that the mothers of children with congenital* Toxoplasmosis may have some abnormal symptoms when pregnant, particularly adenopathy (6 out of 23 cases). Lymph node swelling appeared between the fourth and seventh months, some still persisting five months after delivery. There is no doubt that in such cases the diagnosis could have been established during pregnancy and therapeutic measures taken.

SUMMARY

The author reports the results of biological investigations for Toxoplasmosis carried out at the Clinique de Puériculture in Paris. Good correlation is found between the *serological tests and inoculation experiments*.

Clinical observations agree with *Siim's* description of toxoplasmic lymphadenopathy.

abdominal crisis, appendicitis was suspected by the family physician. A laparotomy performed on November 25, 1954, showed a normal appendix and enlarged mesenteric lymph nodes Although the lymphadenopathy was much larger than is usual in similar cases, no biopsy of the lymph node was made. On the same day a high fever started, oscillating around 39° C for ten days, although there was no apparent post-operation complication. After ten days, this high fever gave place to a continuous sub-febrile state (38° C), with pallor and asthenia, which lasted two months. Tuberculin tests and salmonellosis serological reactions were performed and remained negative.

When first seen by us, two months after laparotomy, she seemed a normal girl in poor general condition. Physical examination showed a single fact. numerous cervical, axillary, inguinal and retrocrural lymphadenopathies. The lymph nodes were firm, well limited, some of them bigger than a cherry. The occipital, epitrochlear and popliteal nodes were normal Spleen and liver normal.

Chest roentgenogram normal Tuberculin tests negative. Sedimentation rate: 1 h· 9; 2 h. 26, 24 h 80 mm. Blood count Hb 90%, R B.C 4,520,000, W B C. 9,700, neutrophil polymorphonuclears 24%, eosinophil 1 5%, lymphocytes 64%, monocytes 10 5%.

Serological reactions for toxoplasmosis were effected on February 2, 1955, two and a half months after the beginning of the illness. Fixation of complement was positive up to 1/128 Dye test was positive up to dilutions between 1/5,000 and 1/10,000 An axillary lymph node, biopsied on February 28, 1955, was inoculated into six mice whose toxoplasmic serological reactions were negative 25 days later, four of the six mice had positive serological tests, and Toxoplasma gondii was demonstrated by sub-inoculation. On April 1955, the patient's serological tests were: complement fixation 1/128, dye test 1/4,000 On October 1955 complement fixation 1/128, dye test 1/1,000.

The child has been observed during the next nine months. Although remaining tired, with moderately enlarged lymph nodes, she had no more abdominal manifestations.

The origin of her toxoplasmic infestation is unknown. She did not drink raw milk and did not eat uncooked meat. During the summer of 1954 she played with dogs

*

Lack of surgical excision of an abdominal lymph node makes the evidence of the toxoplasmic nature of mesenteric lymphadenopathy incomplete. This evidence has been obtained only with the axillary lymph nodes. However, it seems difficult not to be impressed by the close sequence of facts observed in this patient vomiting and abdominal pain, leading to the surgical discovery of important mesenteric lymphadenopathies; immediately after laparotomy, onset of a continuous fever, lasting two months, accompanied by disseminated

Hopital des Enfants-Malades, Paris, France

ABDOMINAL LYMPHADENOPATHY AS FIRST LOCALIZATION OF ACQUIRED TOXOPLASMOSIS

R. JOSEPH, G. DESMONTS,[1] J. C. JOB & J. COUVREUR

Mesenteric lymphadenopathies may be the only surgical finding in a child with an acute abdominal syndrome It is likely that acquired toxoplasmosis, a chiefly ganglionic disease, may realize this special localization. This would support the hypothesis of a digestive contamination in cases of human toxoplasmosis

In fact, this hypothesis has not yet been fully confirmed A brief account has been made by *Diamant-Berger* of two children with abdominal lymphadenopathies and positive dye tests (1) The most important studies on toxoplasmic lymphadenopathy (2, 3, 4, 5) do not mention such cases. We have not found similar reports in a review of the literature devoted to acquired toxoplasmosis (6) before presentation of this paper to VIII International Congress of Paediatries.

This is why we think that the following report may be useful, although not fully satisfying the abdominal lymph nodes not having been excised at time of surgical operation, it has not been possible to give the definite proof of their toxoplasmic infestation by isolation of the parasite. However, the rise in antibody titre and the isolation of toxoplasma from an axillary lymph node make the sequence of facts in this case sufficiently significant to take it into account.

Case History

A six-year-old girl was seen for the first time in Outpatients' Department of the Hopital des Enfants Malades on January 31, 1955, because she remained sub-febrile since a laparotomy performed two months before.

From the beginning of November 1954 she suffered from abdominal pain with vomiting and constipation. She had had in October an unilateral swelling of the parotid region, considered as possible mumps, and the abdominal pain was first interpreted as pancreatitis After three weeks with repeated

1. Laboratoire de recherches sur la Toxoplasmose, Hopital S[t] Vincent de Paul, Paris, France (Head Prof M. Lelong)

and the appearance of superficial lymphadenopathies have been noted after laparotomy. The diagnosis of acquired ganglionic toxoplasmosis has been established by serological tests and confirmed by isolation of Toxoplasma gondii from an axillary lymph node. Since abdominal lymph nodes have not been excised, the initial mesenteric localization of toxoplasmosis is clinically presumed but is not definitely proved.

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superficial lymphadenopathies, whose toxoplasmic origin has been fully demonstrated. This strict chronology seems to come easily under a diagnosis of acquired toxoplasmosis. These clinical arguments, together with some experimental facts, make us admit that the mesenteric adenopathy was the first ganglionic localization of acquired toxoplasmosis in this child.

Experimental facts demonstrated by one of us (G.D.) on the guinea pig led us to suspect the importance of the initial mesenteric localization when the parasite penetrates through the digestive tract. Oral transmission of toxoplasmosis to guinea pig is not possible with vegetative forms of the parasite, which would produce a severe illness if inoculated parenterally, but are inactivated by the gastric juice. On the contrary, if guinea pigs ingest by mouth Toxoplasma gondii cysts, i. e. ground brains of mice suffering from chronic latent toxoplasmosis, the majority of the animals are contaminated. This infestation obtained via the digestive tract is benign The abdominal lymph nodes are then usually infested. Furthermore, it appears that dissemination of Toxoplasma in the whole body is preceeded by its localization in the abdominal lymph nodes. One of us (G.D), by killing the guinea pigs three days after the infesting meal, has isolated Toxoplasma only from the abdominal lymph nodes, the inoculation of other viscera remaining negative. In toxoplasmosis in dogs also, mesenteric adenitis, which may be accompanied by intestinal ulcerations, is not infrequently observed (7).

Although theoretically important, such abdominal ganglionic localizations seem infrequent in human toxoplasmosis. In the Toxoplasmosis laboratory of Hôpital St Vincent de Paul, *M. Lelong, G Desmonts* and *J Couvreur* have started a systematic research for Toxoplasma in abdominal lymph nodes of children laparotomized for acute abdominal adenitis. Up to now, this investigation has been carried out on 22 children although serological tests had demonstrated recent toxoplasmic infection in two of these children, inoculation of the lymph nodes has been negative in all the cases These facts lead us to assume that toxoplasmosis is not a frequent cause of acute abdominal lymphadenopathies, but the limited number of cases under examination forbids any definite conclusion on this point. The present report, as well as a report from *Diamant-Berger*, show that it is worth searching methodically for toxoplasmosis in lymph nodes excised during laparotomies performed because of mesenteric adenitis. Abdominal localizations of toxoplasmosis may be subacute, as in our case, or possibly latent, and then escape examination. Thus their frequency may be difficult to evaluate

SUMMARY

In a six-year-old girl, abdominal pain and vomiting led to laparotomy, which showed abdominal lymphadenopathies. A continuous fever for two months

and the appearance of superficial lymphadenopathies have been noted after laparotomy. The diagnosis of acquired ganglionic toxoplasmosis has been established by serological tests and confirmed by isolation of Toxoplasma gondii from an axillary lymph node Since abdominal lymph nodes have not been excised, the initial mesenteric localization of toxoplasmosis is clinically presumed but is not definitely proved.

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TREATMENT OF TOXOPLASMOSIS

National Institutes of Health, Laboratory of Tropical Diseases,
Memphis, Tennessee, U.S.A.

THE TREATMENT OF TOXOPLASMOSIS

DON E. EYLES

During the years since the discovery by Wolf and his colleagues (1) that *Toxoplasma gondii* is a cause of human disease this parasite has been studied intensively in all its aspects, and numerous reports have appeared describing experimental studies of the chemotherapy of toxoplasmosis in laboratory animals. Also during this period, particularly in the last few years, a smaller number of reports of the use of drugs in man have appeared. The amount of data which has been accumulated up to this time is sufficient to make it desirable to summarize and evaluate the information now available to us.

This summary, which it is our purpose to present to you, should provide a basis for the rational treatment of cases which are met in practice, and should indicate the studies which are needed to evaluate adequately the drugs which are known to have antitoxoplasmic effect. More important, this summary should point out deficiencies in our present knowledge and should indicate problems which remain to be solved in the future.

The Treatment of Experimental Infections.

Because of the paucity of data on the effects of drugs in man, the background for the chemotherapy of toxoplasmosis consists principally of the animal studies which have been reported. For this reason, we shall begin this review with a discussion of the animal work and follow with a summary of the experience in man.

The action of the sulfonamides. The earliest work on the chemotherapy of toxoplasmosis was reported by Sabin and Warren (2, 3) in 1941. These investigators discovered that sulfonamides have definite activity against toxoplasmosis in experimental mouse infections. This activity was independently discovered the next year by Biocca and Pasqualin (4) and subsequently it was shown that sulfonamides were active against this parasite in rabbits (3), pigeons (5), embryonated hens eggs (6), and perhaps other animals. Many investigators in all parts of the world have since confirmed the fact that antitoxoplasmic activity is a general characteristic of the sulfonamide group of drugs, although greatly varying degrees of activity have been described.

A recent paper from our laboratory (7) summarizes the various investigations of sulfonamides and presents data on their relative activity which are essentially in accord with those of others who have used the drugs under comparable conditions (8).

Table I summarizes our work on the relative activity and indicates that sulfamethazine, sulfapyrazine, sulfamerazine, and sulfadiazine are significantly superior to sulfathiazole which is in turn superior to sulfapyridine.

TABLE I

Relative activity of the sulfonamides against experimental toxoplasmosis in the mouse Median effective curative dosages were measured by administering logarithmically related doses in the diet for 14 days Mice were inoculated with about 100 ID50 units and treatment followed just after inoculation (Data abridged from Eyles, 1956 (7))

Name of drug	Median effective curative dosage (as per cent in diet)	95% Confidence limits	
Sulfamethazine	0 027	0 021	0 035
Sulfapyrazine	0 048	0 029	0 079
Sulfamerazine	0 056	0 039	0 081
Sulfadiazine	0 098	0 059	0 164
Sulfathiazole	0 300	0 172	0 522
Sulfapyridine	> 2 000	not estimated	
Sulfadimetine	2 000	not estimated	
Sulfisoxazole	> 2 000	not estimated	

Sulfadimetine [N'-(2,6-dimethyl-4-pyrimidyl) sulfonamide] and sulfisoxazole (3,4-dimethyl-5-sulfanilamidoisoxazole) were also relatively inactive and it is doubtful if their low toxicity can compensate for their low degree of activity.

The use of multiple sulfonamides against toxoplasmosis seems logical and has been reported by Knapp (9) Two experiments in our laboratory confirm the fact that additive action does take place

In our experiments, and in many reported by others, treatment with sulfonamides is frequently followed by relapse and death after the discontinuation of the drug. In our experience animals which have survived for long periods and are apparently cured have been found to be negative to the dye test and completely susceptible to reinoculation Other investigators have reported the establishment of chronic infections after treatment, with resistence to subinoculation (for example, Frenkel (10)). This apparent discrepancy certainly is due to strain differences

In some respects the laboratory mouse is not a favorable host for studying the effect of antitoxoplasmic drugs, as the infection nearly always causes death and the mouse develops little or no effective immunity to most strains of

Toxoplasma. For that reason a more reliable estimate of the possible effect of sulfonamides in man might be gained by studying the effect in the rabbit, as this animal manifests an immune response similar to that of man.

Sabin and Warren (3) and Biocca (11) found sulfathiazole active in rabbits but they did not have at that time the more active, newer sulfonamides. Nobrega and his colleagues (12) in an interesting study later found sulfadiazine effective in checking a spontaneous epidemic of toxoplasmosis in a rabbit colony.

TABLE II

Treatment of experimental toxoplasmosis in the rabbit with sodium sulfadiazine. Rabbits were infected by the intracutaneous injection of 1000 Toxoplasma *organisms. Treatment was initiated either just after inoculation or at the time the fever reached 40° (usually on day 6 of the infection)*

Dosage and day of treament	No rabbits	No survived	Per cent survived	Day of death of dying animals*
500 mg per cent Na SD in water, Rx – day 1	5	4	80	10
1000 mg per cent Na SD in water, Rx – day 1	3	3	100	–
500 mg per cent Na SD in water, Rx – 1st day of fever	7	4	57	*10*, 10, 10
1000 mg per cent Na SD in water, Rx – 1st day of fever	3	1	33	10, 18**
Untreated controls	9	0	0	*9, 9, 9, 9, 9, 9, 9, 10, 10*

* If *Toxoplasma* were found at autopsy, this is indicated by italic numerals

** This animal probably did not die of toxoplasmosis

In our laboratory we have obtained apparent cure of rabbit toxoplasmosis by the oral administration of sodium sulfadiazine in the drinking water. These experiments are summarized in table II The rabbits were inoculated intracutaneously and treatment was initiated either immediately after inoculation or after the appearance of fever over 40° C. (usually on the 6th day of the infection). The dosages used produced blood levels of the order of four to eight mg per 100 ml. of blood.

It can be seen that when the drug was started immediately, most of the animals were cured Skin lesions failed to develop in treated rabbits or developed only poorly. When treatment was delayed until fever had appeared only five of ten animals were cured but autopsy failed to reveal *Toxoplasma* in some of the rabbits which died The control rabbits died uniformly in from nine to 10 days showing typical skin lesions and abundant *Toxoplasma* in visceral smears.

All of the cured animals were sacrificed about six weeks after treatment

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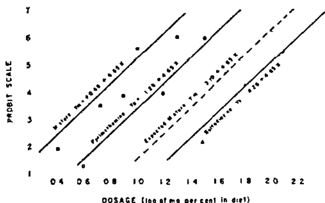


Figure 1

Dose-effect curves pyrimethamine and sulfadiazine administered alone and in combination The broken line represents the expected effect of the mixture The displacement of the observed mixture curve represents graphically the degree of synergistic effect.

compared with 0.098 for sulfadiazine. Although clearly superior on a gram for gram basis, the curative dosage of pyrimethamine approaches the maximum tolerated level, whereas, sulfadiazine gives maximum therapeutic effect well below the toxic level.

Synergistic action of pyrimethamine and sulfonamides. Soon after the activity of pyrimethamine was discovered it was found that this drug acts synergistically with sulfadiazine (17) and recently we reported much more detailed observations on this subject (16). That synergistic action takes place has been confirmed by Beverley (18) and by Jacobs *et al* (19)

In our experience, we have achieved better therapeutic results with combinations of these two drugs than with any other drug or combinations of drugs. Cure is frequently obtained even when inocula of up to 100,000 ID50 units are used and nearly all mice are cured when small inocula are employed The dosages which produce cure in mice are sufficiently low to indicate that the drugs might be effective in man at non-toxic levels For example, using a dosage of sulfadiazine which produced levels easily attainable in man, we obtained cure in most mice given a dosage of approximately 1 1 mg per kg. per day. This would correspond to a dosage in man of about 70 mg per day but perhaps levels similar to those attained in the mouse would be obtained from lesser dosages in the larger host

The degree of synergism between pyrimethamine and sulfadiazine was very great as is evidenced by figure 1 which plots observed and expected dose-response curves for the drugs alone and in combination following the methods developed by Finney (20) Calculations showed that the observed potency of the mixture was more than six times that which would result from additive action alone. Still following Finney, a coefficient of synergism (K) of + 10.2

and tissues subinoculated into uninfected laboratory mice. None developed infections indicating that eradication of the parasites had occurred. This is in contrast to the observation of Nobrega *et al.* (13) who found that 90 per cent of the rabbits which survived for 15 to 36 months after the treatment of spontaneous toxoplasmosis showed parasites in the brain.

Owing to a lack of facilities for maintaining rabbits we have discontinued these promising experiments pending the availability of adequate animal quarters.

TABLE III

Dose-effect chart comparing the activity of pyrimethamine and sulfadiazine on a gram for gram basis. Treatment was initiated just after the intraperitoneal inoculation of 20,000 organisms, and was continued for 14 days

Dosage (as per cent of diet)	Pyrimethamine		Sulfadiazine	
	No mice	Per cent cured*	No. mice.	Per cent cured*
0 125	–	–	18	67
0 063	12	75	18	22
0 031	18	83	18	0
0 016	18	11	12	0
0 008	18	6	12	0
0 004	12	0	–	–

* Surviving mice were considered cured if blind passage of the tissues to uninfected mice failed to reveal *Toxoplasma*

The sulfonamides probably have the same mode of action against *Toxoplasma* as against other pathogenic organisms. Summers (14) has shown that *p*-aminobenzoic acid antagonized the effect of sulfathiazole and that folic acid had a similar inhibitory effect. This would indicate that the sulfonamide competes with the metabolite, *p*-aminobenzoic acid, thus interfering with the cellular metabolic processes leading to the synthesis of folic acid.

The action of the 2,4-diamino pyrimidines. In 1952, our laboratory reported that pyrimethamine (2,4-diamino-5-p-chlorophenyl-6-ethyl pyrimidine; the trade name of Burroughs Wellcome Corporation is Daraprim) was found to be very active against toxoplasmosis in the mouse. This activity was independently reported by Summers (15) and has been confirmed by other workers. Other members of this same group of compounds also were found to be active but pyrimethamine is the most active yet tested.

In a later report (16) our laboratory presented an evaluation of the curative effects of pyrimethamine as compared with sulfadiazine and reached the conclusion that the former was superior when considered on a gram for gram basis. Table III is illustrative of this point, as it can be seen that the median effective curative dosage of pyrimethamine is 0.023 per cent in the diet as

The explanation of the strong interaction between pyrimethamine and the sulfonamides probably is that these drugs produce sequential blocking of the metabolic pathway which leads to the synthesis of folinic acid The role of the sulfonamides as antagonists of *p*-aminobenzoic acid is well known and pyrimethamine has structural similarities to folic acid. The work of Hitchings and his colleagues (25, 26) deals most thoroughly with the theoretical aspects of this problem and the papers cited should be consulted by anyone with an interest in this field.

TABLE IV

Relative activity of sulfones against experimental toxoplasmosis in the mouse Median effective dosages for 14 day survival of several sulfones and sulfadiazine showing relative activity. Intraperitoneal inoculation af about 1000 ID50 units was followed immediately by 14 days treatment with drugs in diet suspension

Name of drug	Median effective dosage 14 day survival (as per cent in diet)	95% Confidence limits	
4,4'-diaminodiphenyl sulfone	0 009	0 006	0 013
Promacetin	0 038	0 030	0 049
Promin	0 110	0 082	0 148
Diasone	0.230	0 135	0.391
Sulfadiazine	0 098	0 059	0.164

Other drugs active against toxoplasmosis. Other than the drugs discussed above, only the sulfones and the antibiotics are sufficiently active to require discussion in detail.

Sulfones were found to be active by Biocca (27) and subsequent to the first report he and his coworkers carried out a series of studies of various compounds of this group. Activity of sulfones was confirmed by Summers (28) and later by a number of other investigators. Beverley *et al* (29) used 4,4'-diaminodiphenyl sulfone in the treatment of experimentally induced, acute uveitis in rabbits, the beneficial effects being augmented by the use also of cortisone

It is difficult from reviewing the literature on the sulfones to determine their relative activity and how their activity compares with that of the sulfonamides. Because of this difficulty, we have tested under uniform conditions in our laboratory as many of the sulfones as could be obtained. Unfortunately, we were unable to obtain some of the more active ones used by Biocca.

Table IV, which is adapted from a paper from our laboratory (75) summarizes data on several of the sulfones and compares them with sulfadiazine. This table presents median effective dosages based on survival through the treatment period (median effective curative dosage was not used because a number of the sulfones did not cure sufficient mice to

was calculated. This constant, which is a measure of the degree of interactions of the drugs, may prove useful in the future for the comparative study of drug combinations.

Our most intensive studies have been of the interaction of pyrimethamine and sulfadiazine, but we have also used pyrimethamine with sulfapyrazine and sulfathiazole With these combinations synergism could also be demonstrated with ease, but the degree of interaction may have differed

The outstanding action of combinations of pyrimethamine and sulfadiazine in mice led us to try these drugs in other hosts. These experiments although incomplete have disclosed some interesting facts.

In albino Norway rats inoculated intraperitoneally, pyrimethamine and sulfadiazine eradicated the infection in most of the animals treated even when treatment was delayed until one week after inoculation (21). The effect of treatment was assessed on the basis of the proportion of rats from which parasites could be recovered by subinoculation because Norway rats nearly always survive infection whether treated or not. On the other hand, when treatment was delayed until a month or more after infection, the use of pyrimethamine and sulfadiazine did not result in eradication, for *Toxoplasma* could be recovered as readily from treated as from untreated rats. These experiments, which involved a large number of rats treated with a number of regimens, indicate that these drugs have no action on the chronic form of the parasite, presumably the pseudocyst.

Pyrimethamine and sulfonamides have also been used in puppies experimentally infected with *Toxoplasma*. In four instances, *Toxoplasma* could not be recovered from treated puppies; whereas, three of four untreated littermates could be shown to be infected by subinoculation.

Finally, we have used combinations of pyrimethamine and sulfadiazine in the treatment of week-old chicks infected by intracerebral inoculation. Survival of a significantly larger proportion of chicks resulted from the treatment. In the chicks which survived, chronic infection was much less prevalent in those which had been treated than in the non-treated ones.

From the above it is evident that the combination of pyrimethamine and sulfonamides is effective, to some degree at least, in a variety of hosts. Beverley (18) using sulfones instead of sulfonamides and Jacobs (19) have found the joint treatment effective in rabbits and Cook *et al.* (22) demonstrated synergism in roller tube tissue cultures.

Synergistic action of sulfonamides and compounds related to pyrimethamine have been described with other parasites; in fact, the experiments with *Plasmodium gallinaceum* reported by Greenberg and Richeson (23) directly stimulated our own studies. In addition Lux (24) has shown that sulfonamides and diaminopyrimidines act synergistically against *Eimeria tenella*.

group makes it essential that continued study be given these substances as they become available.

Another group of compounds of theoretical interest at least are the dihydro-s-triazines which have been studied by Winters and Foley (32). These drugs, which have antifolic acid properties, are active against *Toxoplasma* and act synergistically with sulfonamides, but insufficient information is available to evaluate the effects on a comparative basis

Space will not allow a review of the large number of compounds which have been found to possess very slight antitoxoplasmic activity and the very large number found to possess none Eyles (33) presented some data on the minor drugs in a previous review and Eyles and Summers (34) have published summaries of screening studies done in their laboratories.

Suffice it to say that only one generalization has been reached and that is that antimalarial activity is often associated with antitoxoplasmic activity. Illustrative of this general finding is the activity against both parasites of pyrimethamine, sulfonamides, sulfones, compounds related to paludrine, some quinolines, and the napthoquinone, Lapinone. Further work is necessary to determine how consistently this parallel in activity occurs.

The Treatment of Toxoplasmosis in Man.

Congenital infections. Treatment has been attempted in a number of cases of congenital toxoplasmosis, but the results are very difficult to evaluate. The reasons for this are several fold In the first place, diagnosis often comes after irreversible changes have taken place which drugs could not remedy. Secondly, when treatment is administrated prognosis is so uncertain that effects of treatment can scarcely be assessed in the absence of control series, and cases of toxoplasmosis are so infrequent as to make the establishment of controls impracticable.

Even so, drugs have been used in a number of congenital cases and the results can be reviewed although no positive conclusions are warranted. Possibly other cases have been treated of which I am unaware.

Sulfonamides have been used most frequently Dow (35) treated one five year old child with sulfapyridine with some possible temporary improvement Jacoby and Sagorin (36) also used sulfamethazine to control respiratory infections in a case and thought it possible that a favorable effect upon the toxoplasmosis infection could be observed. Nelson and Mantz (37) gave a four month old patient massive dosages of triple sulfas (sulfadiazine, sulfamerazine, and sulfathiazole) for several months and believed that improvement might have been due to the treatment. Verlinde and Makstenieks (38) also treated a case using sulfamerazine (sulphamethylpyrimidine) but isolated organisms from the spinal fluid and blood in spite of the treatment.

Sabin *et al.* (39) give a reference to the successful treatment of half of a

allow reliable estimates to be made). It can be seen that only 4,4'-diaminodiphenyl sulfone has a lower MHD than sulfadiazine, and this drug is much more toxic than the sulfonamide. All of the other sulfones are much inferior with Promacetin and Promin being superior to Diasone.

Other sulfones tested included Sulphetrone and Rhodilone. The former showed only slight activity when given in enormous dosages and the latter was inferior to 4,4'-diaminodiphenyl sulfone. In general in our work, antitoxoplasmic activity was greatest in those compounds which would degrade most readily to 4,4'-diaminodiphenyl sulfone; however, a few compounds which could not by virtue of their structure thus degrade were significantly active.

The conclusion reached was that of the sulfones tested only 4,4'-diaminodiphenyl sulfone was sufficiently active to be of possible practical value. Since it is a very toxic drug, its use should perhaps be confined to subjects unable for some reason to tolerate the active sulfonamides. We disagree emphatically with the opinion of Cross (30) that Diasone and Promin are more active than sulfadiazine.

The mode of action of the sulfones is undoubtedly similar to that of the sulfonamides for we have found that synergism takes place between pyrimethamine and 4,4'-diaminodiphenyl sulfone and between pyrimethamine and Diasone.

Antiobiotics have been extensively tested against experimental toxoplasmosis and the most active yet tested are probably chlortetracycline (aureomycin and spiramycin) (77). Oxytetracycline (terramycin), tetracycline, chloromycetin, fumagillin, erythromycin, puromycin, and magnamycin, have also been reported to possess some activity.

Basing their conclusion mainly on studies of toxoplasmosis in rabbits, Giroud and Gaillard (31) are of the opinion that oxytetracycline is more active than chlortetracycline in rabbits and that it may be a drug of practical importance, but studies with the mouse do not support this conclusion.

In our laboratory we have recently conducted experiments with puromycin, the aminonucleoside of puromycin, and several other analogues. Most of these possessed antitoxoplasmic activity and the aminonucleoside was much more active than the parent compound (just as against *Trypanosoma* but differing from the effect against *Endamoeba*) Also it was observed that the purine metabolite, adenine, at least partially reversed the effect of puromycin, thus indicating that the action of the drug was possibly in some way related to the utilization of this metabolite by the parasite.

A recent paper from this laboratory (7) reviews the other work on the antibiotics and the conclusion was reached that none of the antibiotics was sufficiently active to be of practical importance in the treatment of toxoplasmosis. In spite of this, the fact that activity is widespread in the antibiotic

tetracycline failed in the case of Kass (42) and the case of Sexton *et al.* (43) just as did sulfadiazine. Some German scientists (50) have reported that this drug causes a disappearance of dye test antibody, but Frenkel *et al.* (51) and others could not confirm this finding.

Meira *et al.* (52) used chloramphenicol in a case first suspected of being typhoid fever and reported favorable effects, and Michel *et al* (53) used erythromycin in a case with apparent good effect. Neither of these cases was proved by the finding of parasites, and the serologic evidence upon which the diagnosis was based was not entirely convincing particularly in the first case. Biocca and Grieco (54) stated that they treated a case with 4-nitro-4-formylaminodiphenyl sulfone with complete success, but provided none of the details necessary for critical evaluation. Scotti (55) treated a case with sulfonamide, antimony and chlortetracycline with success and Rauscher (56) used oxytetracycline to produce regression of both clinical signs and serologic titers. These last two cases are cited from abstracts and have not been studied in detail Undoubtedly there are other cases of which I am not aware.

The occurrence of two laboratory cases during the past two years presented the opportunity of testing the action of pyrimethamine and sulfonamides in combination against acute adult toxoplasmosis One of the cases occurred in our laboratory (57) and the other in the laboratory of Dr. Jacobs (58) to whom I am indebted for information

In the case from our laboratory, the principal symptoms were fever, malaise and lymphadenopathy and the patient was growing progressively worse until the initiation of treatment with pyrimethamine and triple sulfonamide Following treatment, which was initiated just as a rash appeared, the patient became fever-free within 24 to 36 hours and other symptoms promptly abated The clinicians observing the patient were unanimously of the opinion that a therapeutic response had occurred Convalescence was slow but uneventful. The diagnosis was proved by the isolation of parasites from an excised lymph node and from blood The regimen of drugs used consisted of an initial dose af 50 mg. of pyrimethamine followed by 25 mg in six hours and 25 mg per day thereafter for two weeks along with 6 g per day of a triple sulfonamide preparation containing equal parts of sulfadiazine, sulfamethazine, and sulfamerazine.

In the second case, which was similar in most respects to the first the same regimen of treatment was used and recovery has resulted, however, the patient has not been observed sufficiently long to allow complete evaluation of the results of treatment This case also was proved by the recovery of parasites.

Petrovicky (59) in Czecho-Slovakia also recently reported the cure of a case of acute toxoplasmic meningoencephalitis in an adult with pyrimethamine and sulfadiazine but this case was not proved parasitologically. Dr. Petrovicky has informed us that he has also been successful in other cases (60).

series of 12 infants with sulfonamides by Eichenwald, but further details regarding these cases have not been published. In another paper (40) Eichenwald and Levine state that experience with sulfonamides combined with serum therapy has been moderately encouraging.

So far as we know, the combination of pyrimethamine and sulfonamides has been used in only a few cases of congenital toxoplasmosis. Large dosages were used in an advanced case studied by our laboratory, but the child, which was gravely ill when treated at the age of two weeks, died in spite of the therapy. Eichenwald (41) has used these drugs in ten cases of congenital toxoplasmosis. It appeared, that the course of the acute illness was probably shortened both by this treatment and by the sulfonamide and serum therapy. Perhaps the future will bring further reports, but the very nature of the congenital disease so limits the possibilities of treatment that the prospect is not encouraging.

The treatment of acute, acquired infections. More evidence is available regarding the treatment of acute, acquired infections in man, but again evaluation must be made cautiously due to the unavailability of controls.

Sulfonamides have been used in several cases Kass *et al.* (42) used sulfadiazine in one fatal case and this drug also failed to check the course of a fatal infection in a laboratory technician of our laboratory (43). However, in the case of Kass a chronic rather than an acute infection may have been involved and in the laboratory case treatment was started so late that a fair trial was not afforded.

Sabin (44) treated a case of encephalitis due to toxoplasmosis with sulfanilamide during the final week and did not avert a fatal outcome although the fever of the patient abated somewhat for two days after treatment. Beverley *et al* (45) used sulfamethazine in a non-fatal laboratory acquired infection and the patient became fever-free within five days, but the authors stated that no conclusion as to the effect of the treatment could be drawn. Strom (46) also used sulfadimine in a laboratory case but drew no conclusions as to the effect.

On the other hand, Hormann (47) treated a laboratory infection with the sulfonamide preparation, supracid (a mixture of sulfamerazine and globucid) and concluded that the recovery of this patient without complications was due to this drug, and earlier, Franke and Horst (48) had reported good results in several cases treated with the sulfonamide preparation solusupronal which also contains sulfamerazine Likewise, Robinson (49) reported successful treatment of a peculiar case of meningoencephalitis with chorioretinitis with sulfathiazole and emetine In none of these cases was *Toxoplasma* recovered in laboratory animals, although organisms were reported seen in spinal fluid smears or in smears from skin lesions in all except the case of Hörmann.

Antibiotics have been used in a few cases of acquired toxoplasmosis. Chlor-

tetracycline failed in the case of Kass (42) and the case of Sexton *et al.* (43) just as did sulfadiazine. Some German scientists (50) have reported that this drug causes a disappearance of dye test antibody, but Frenkel *et al.* (51) and others could not confirm this finding.

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Siim and his colleagues (61) treated a severe case of toxoplasmosis in an ıdult with pyrimethamine and sulfonamides. The diagnosis was based on ırefully conducted serologic studies through the course of the disease. This ·cent case is still under study.

From the above instances in which drugs have been used for the control f acute, acquired toxoplasmosis, it is difficult to draw firm conclusions. The pparent response to pyrimethamine and sulfonamides in two parasitologi- ılly proved cases is at least encouraging, and there would appear to be some ısis for suspecting that the sulfonamides alone have an effect; however, ıal conclusions must await observation of many more cases.

he treatment of toxoplasmic uveitis and chlorioretinitis.

Results of treatment of granulomatous uveitis or chlorioretinitis due to ›xoplasmosis have been slow to appear due to the great difficulty of diag- ›sis and the difficulty of assessing the results; however, the results of a few udies have become available in the past few years and others are in progress.

In 1951 and 1952 several Scandinavian scientists (62, 63, 64) reported ıccessful treatment of chorioretinitis presumed due to *Toxoplasma* with ebrin and with atebrin plus plasmoquine. These compounds have little : no effect against acute infections in laboratory animals, but their possible fect upon chronic stages should be investigated.

Pyrimethamine and sulfonamides have been used in most of the other ıdies. The first series studied was that of Ryan and his associates (65) who und that 25 of 29 cases presumed to represent toxoplasmosis showed im- ovement suggesting favorable response to the therapy. Eight responded ithin a week and seventeen within a month Some of the cases had been ıder treatment only a short time at the time of the report and only one case ıpeared to represent failure of the treatment

Ryan's group reported data on toxic side reactions and cautioned against e use of the drugs except under careful medical supervision. A number of gimens were used including pyrimethamine at up to 100 mg. per day, but › correlations between dosage and effect were made.

Cassady *et al.* (66) in 1955 reported good results in most of a series of 11 ıtients treated with 25 mg per day of pyrimethamine per 60 pounds of ›dy weight (about 27 kg.) plus 1 g. of sulfadiazine per 60 pounds per day. ›me of the patients exhibited renewed activity after discontinuation of the ›atment and in one of the patients severe depression of leucocyte and atelet formation took place.

More recently, Hoover *et al.* (67) reported on the treatment of 22 cases presumptive ocular toxoplasmosis and observed improvement in 13 in- ıding four in which excellent results were achieved There was no improve-

ment in six cases and three were doubtful. The regimen used was 25 mg. per day of pyrimethamine along with sulfonamides. In spite of the lower dosage, two cases of leucopenia developed which were sufficiently severe to require treatment by transfusion.

In Memphis, our laboratory is cooperating with several ophthalmologists in a study of the prevalence of ocular toxoplasmosis and in its treatment. A number of patients have shown marked improvement; one case has been reported (68). The regimen used most frequently has been 50 mg. per day of pyrimethamine plus the normal dosage of sulfadiazine or triple sulfonamide for two or more weeks. No toxic reactions have been noted up to this time.

Additional reports on the use of pyrimethamine and sulfonamides continue to appear (71, 72, 73) and only Hogan failed to achieve at least a degree of success. The only adequately controlled study of the treatment of uveitis is that of Perkins *et al.* (74) who used pyrimethamine alone In their series uveitis patients were treated irrespective of immunologic status and a significantly larger proportion of those with positive toxoplasma reactions improved under therapy.

The reports cited strongly indicate that pyrimethamine and sulfonamides are effective against toxoplasmic uveitis and chorioretinitis. Undoubtedly misdiagnosed cases were included in the treated group as the diagnosis of ocular toxoplasmosis is always presumptive and based in part at least upon a process of elimination of other possible causes. Paradoxically, success is apparently being obtained even though the drugs would not be expected to exert an effect upon the chronic pseudocyst stage Possibly the drugs act by controlling the foci of active proliferation. These drugs would not be expected to act if the inflammation was a result of pseudocyst rupture as is postulated by Frenkel (69) If the action is upon actively multiplying parasites one might expect recurrence of activity originating from pseudocysts remaining after treatment.

Before leaving the subject of the treatment of ocular toxoplasmosis, emphasis should be placed on the fact that the dosages of pyrimethamine used for this purpose have in some of the studies approached levels toxic to man. Three serious reactions have been described in the 70 or more cases summarized. The treatment should be done only under careful medical supervision, and frequent blood studies including white cell, red cell, and platelet counts are essential Myatt *et al.* (70) found that although the administration of 25 mg. of pyrimethamine per day over long periods sometimes resulted in a megaloblastic anemia, this anemia disappeared promptly after discontinuation of the drug. Studies in our laboratory using mice showed no interaction between pyrimethamine and sulfonamides in the production of acute or chronic toxicity (16).

Recent investigations by this laboratory and by Frenkel and Hitchings (76)

have shown that folic acid administered orally to mice treated with combinations of pyrimethamine and sulfadiazine had no effect upon the action of the drugs. It is possible that this vitamin may be used in man to counteract or prevent toxic effects without interference with the therapeutic effect.

DISCUSSION

This discussion can best take the form of comments on the defects in our present knowledge of the treatment of toxoplasmosis. Screening of new drugs for toxoplasmosis should be continued for it is far from certain that pyrimethamine and sulfonamides constitute adequate drugs as their effect is limited to proliferating parasites and pyrimethamine at least must be used at a dosage close to the toxic level. Certainly new antibiotics and other drugs active against other organisms should be tested as they become available.

More important, there is no experimental evidence that any drugs are active against the pseudocyst stage of the parasite Furthermore, very few programs of drug study have been designed to find drugs active against this form, but instead research has been concentrated on the treatment of acute infections, mostly in the mouse. Drugs active against the pseudocyst stage would be extremely useful in the treatment of chronic forms of toxoplasmosis such as the ocular manifestations, and would probably be useful also in the treatment of sub-acute phases as in congenital toxoplasmosis.

Additional experimental work should be done to determine the effect of drugs in hosts other than the mouse, and more attention should be given to the use of drugs late in the infection after symptoms have appeared rather than just after inoculation Fundamental work on the mode of action of drugs and their relationship to metabolically active substances should be continued, for such studies should provide basic physiological information as well as information important in the development of chemotherapy.

Most important of all, much more information is needed on the activity of drugs in man. Parallel to this need, improvement and more skillful use of the methods of diagnosis is necessary so as to allow certain and early detection of cases. Many cases are recognized so late that treatment is of little avail, and treatment is often applied in cases in which the diagnosis is uncertain or in error.

The paucity of information is most marked in the case of the treatment of congenital toxoplasmosis At present it can only be recommended that subjects be treated with those drugs which are most active in animals; that is, pyrimethamine and sulfonamides in as large doses as are safe. Perhaps three to six mg. of pyrimethamine per day plus the usual sulfonamide dosage would be proper for very young infants, and treatment for two weeks, repeated if

necessary, is advisable. It is to be hoped that physicians working in large centers, where cases are seen relatively frequently, will be able to reach some conclusions as to effect.

More information is available on the treatment of acute, acquired toxoplasmosis, partly because of the occurrence of a number of laboratory cases. There would appear to be some evidence that sulfonamides and especially pyrimethamine and sulfonamides exert some action, but much more evidence is needed. Again, the only recommendation possible is the use of maximum dosages of pyrimethamine and sulfonamides; that is, 25 to 50 mg. per day of pyrimethamine plus four to eight grams per day of one or a combination of the most active sulfonamides for two weeks. For children, the dosage should be scaled down in proportion to the weight.

The treatment of ocular toxoplasmosis presents an opportunity for obtaining evidence more scientifically. The studies so far, though reporting success with pyrimethamine and sulfonamides, are subject to the valid criticism that no control groups were maintained parallel to the treated groups Cases of this form of the disease are sufficiently frequent that workers in the larger centers might be able to accumulate series of cases which would answer unequivocally the question as to whether or not treatment is beneficial.

SUMMARY

A review of the literature on the treatment of toxoplasmosis together with a summary of the experience in our laboratory has been presented The available evidence indicates that the drugs which are most effective against toxoplasmosis in laboratory animals are pyrimethamine and the sulfonamides (particularly sulfamethazine, sulfapyrazine, and sulfadiazine) The action of these compounds individually is overshadowed by their outstanding synergistic action. By the joint administration of these drugs, good results can be obtained in the treatment of toxoplasmosis infections in animals at dosages of a magnitude that can be used safely in man.

The evidence regarding the chemotherapy of toxoplasmosis in man is unsatisfactory. Because of the variability of the severity of infections, evaluation is very difficult, but there is some evidence that pyrimethamine and sulfonamides are effective, particularly in the case of eye infections

A discussion of the deficiencies in the present knowledge of the treatment of toxoplasmosis is presented, and recommendations for management based on the evidence now available are given.

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OPHTHALMOLOGICAL ASPECTS OF TOXOPLASMOSIS

U.S Department of Health, Education, and Welfare, Public Health Service,
National Institutes of Health, National Institute of Allergy and Infectious Diseases,[1]
Bethesda, Maryland, U.S.A.

OCULAR TOXOPLASMOSIS· LABORATORY CONTRIBUTIONS TO DIAGNOSIS AND CHEMOTHERAPY

LEON JACOBS

The frequency of ocular lesions in congenital toxoplasmosis is so high that chorioretinitis is very often the clue to diagnosis Feldman (1953), for instance, reports chorioretinitis in 99% of the neonatal cases he has studied; and Eichenwald and Levine (1954) report that about 80% of cases show this symptom. Of considerable importance is the observation of the latter authors that, in some cases of congenital toxoplasmosis, ocular lesions do not appear until several months to even years after birth. In a few cases, this has been the only residuum of congenital infection. Binkhorst (1948) mentioned, also, a case of congenital toxoplasmosis in which acute chorioretinitis reappeared at age 5 years Thus, in the congenital disease, chronic ocular infection may develop.

There is also no doubt that adult ocular toxoplasmosis does occur. The demonstration, by Wilder (1952) of organisms resembling *Toxoplasma* in eyes enucleated because of chorioretinitis, together with the serological identification of these parasites as *Toxoplasma* (Jacobs, Cook, and Wilder, 1954) and the isolations of this parasite from an enucleated eye by Jacobs, Fair and Bickerton (1954) and from subretinal fluid by Habegger (1954) leave no doubt that this ocular disease does occur The question of its frequency, which is dependent on diagnostic means, will be discussed below.

The view has been expressed that these adult cases might conceivably represent the residua of congenital toxoplasmic infection Franceschetti and Bamatter (1953) raise this point, and cite cases studied by Duke-Elder *et al*, Bohn and Koch (1951) and others, which might be illustrative of such conditions. The bulk of cases of acute toxoplasmosis in adults (Sim, 1956, Gard and Magnusson, 1951; Kass *et al*, 1952; Brown and Jacobs, 1956) have revealed no ocular lesions However, one case reported by Wising (1952) provides evidence that ocular lesions can develop in acquired toxoplasmosis. The case in question suffered a brief episode of a syndrome resembling infectious mononucleosis with, however, a negative heterophile reaction and with the development of high titers in the dye test for toxoplasmosis. Although toxoplasmas were not demonstrated directly, the serologic evidence favors

1. Laboratory of Tropical Diseases

TABLE I

Serologic test results and other data on patients with histopathologically diagnosed toxoplasmic chorioretinitis

AFIP. ACC. No.	Preoperative history, duration of symptoms	Unilateral or bilateral	Age at tests	Time from enucleation to serology	Dye test	C f test	Cerebral calcification
289604	6 mo	U	16	2 yr.	1 64	Neg.	Neg.
180418	9 mo.	B*	25	7 yr	1 16	Neg	
313108	7 mo.	U	25	3 yr	1 16	Neg	
482589	4 mo.	U	28	1 yr	1 16	Neg.	
161182	3 mo	U	28	7 yr	1 16	Neg	Neg
298310	4 mo	U	29	2 yr	1 4	Neg	Present
281891	1 yr 2 mo	B*	33	3 yr	1 64	Neg	Neg
541058	3 yr	U	35	3 mo	1·16	Neg	
84822	2 mo.	U	38	11 yr.	1 16	AC	Neg
130487	6 mo.	B	38	8 yr.	1 2	Neg	
551423	8 mo	U	41	3 mo.	1 32	Neg	Present
154482	10 mo.	U	42	8 yr	1 16	Neg	
238453	1 yr. 3 mo	U	45	3 yr	1 2,048	Pos	
165904	5 mo	U	44	6 yr	1 64	Neg	Neg
95175	4 mo	U	44	10 yr	Undil	Neg	
563586	2 yr.	U	59	4 mo	1 32	Neg	
493829	7 mo	U	66	8 mo	1 64	Pos	
281886	6 mo.	U	66	2 yr	1 32		
286700	6 mo	B*	70	3 yr.	1 32	Neg	
162235	9 mo.	U	77	5 yr.	1 256	Pos	
598327	4 mo.	U	38	1 mo	1 256		

* This indicates that the lesion in the remaining eye was old and healed

1.) An ocular lesion may become apparent during, or very shortly after the appearance of systemic symptoms (the cases of Wising and NIH).

2.) An ocular lesion may develop during this period, but not be observed because of a peripheral location in the fundus, and because the subjective symptoms produced are so minor that attention is not called to the eye.

3.) Toxoplasmas may localize in the retina during the acute stage of the infection, which may be symptomatic or asymptomatic, but fail to reproduce to any extent

Under any of these circumstances, it is postulated that proliferation of parasites in the eye is soon retarded by the elaboration of high levels of antibody. Thus, whether or not the localization of the parasite in this site is apparent at first, some residual organisms lying dormant in the retina can later rupture their cysts and renew proliferation This may occur when serum antibodies decline to such low levels that their concentration in the eye, always relatively much lower than in the serum, because of the blood-ocular barrier, becomes negligible. This is the postulated basis for chronic, recur-

the diagnosis. A juxtamacular chorioretinitis appeared about 1 week after the febrile episode.

Another case of lymphadenopathic toxoplasmosis has recently been under study at the National Institutes of Health in Bethesda, Maryland. In this case, the parasites were demonstrated by inoculation of mice, chick embryos, and tissue cultures with material obtained from various biopsies of lymph node and muscle. The complete report on this case will be published elsewhere. The information concerning it which is pertinent to our present thesis is that this case also developed a chorioretinal lesion. The patient first complained of blurring of vision about 1 month after the onset of systemic symptoms. She was not seen by an ophthalmologist until 4 or 5 months after her first symptoms, at which time a small peripheral lesion was seen in one eye This lesion was active. It appeared arrested after treatment with pyrimethamine and sulfadiazine.

Admittedly, neither of these cases furnishes *absolute* proof that the chorioretinal lesions were indeed due to *Toxoplasma*, and not merely coincidental in time with the systemic disease. However, it is believed that the coincidence is presumptive evidence in favor of the same etiology, especially since in the NIH case there was a clinical response to therapy.

In addition to these data, we have the information on the patients in whose eyes Wilder demonstrated *Toxoplasma* and whose serum was positive in the dye test for toxoplasmosis. It is to be noted (Table I) that some of these patients were 65 years of age or over before their ocular symptoms appeared. We do know that *Toxoplasma* may survive for years in animals and has also been found in the brain of a case of toxoplasmosis 3½ years after birth (Desmonts) and in the eye of a man with a history of chorioretinitis of 8½ years' duration (Jacobs, Fair, and Bickerton). However, despite these long survivals of *Toxoplasma* within its hosts, it nevertheless seems a most implausible assumption that the parasites survived 65 years or more before producing ocular lesions

Theoretically, the concept that congenital toxoplasmosis differs from the acquired disease in that the former always involves the central nervous system, while the latter affects the extraneural viscera, is responsible for doubts concerning ocular involvement in the latter type. However, while the symptomatology of acquired toxoplasmosis may indicate this view, nevertheless encephalopathy has been found in a number of acquired cases (Pinkerton and Henderson, *1941*; Strom, *1951*; Sexton *et al*, *1953*). The distinction between acquired and congenital toxoplasmosis is a matter of degree rather than of kind.

During the acute stage of the infection, parasites can be carried throughout the body in the blood stream. It is believed that, fortuitously, they can on occasion localize in the eye. Under such circumstances, it is postulated that any of the following events may occur:

TABLE II

Statistical data on the relation between immunologic tests for toxoplasmosis and chorioretinitis

Author	Date	Test used	Total cases	Chorioretinitis		Controls	
				No	% Pos	No	% Pos
Frenkel	1951	Intradermal		28	71.0	90[1]	20.0
Hogan, Thygeson	1952	Intradermal		125	21.6	201[2]	9.0
& Kimura		Dye		125	43.2	201[2]	21.9
Hogan	1955	Dye	644		48.0		23.0
Busacca, Nobrega & Giovannoni	1952	C f		35	28.5	100	3.0
Woods, Jacobs Wood & Cook	1954	Dye		191[3]	40.3	208[1]	24.5
Smith & Ashton	1955	Dye		31	67.7	40[2]	39.2
		C f		31	28.2	40[2]	7.8
Siegert	1955	C f		339	27.1	822[4]	14.4
Keller & Vivell	1952	Dye		17	41.0	80	14.0

[1] Includes both non-uveitis and anterior uveitis cases, since the latter show percentages of positives similar to "normal" controls and are not thought to be due to infectious agents.

[2] Anterior uveitis cases

[3] Excluding 10 cases of recognized congenital toxoplasmosis included in original tabulation.

[4] All kinds of ocular disease other than granulomatous

reactions in the group with ocular disease classified as granulomatous uveitis. (This term is used to differentiate inflammation of the posterior portion of the eye, due to infectious agents, as contrasted with non-granulomatous uveitis, which is generally anterior and is believed to be due to allergic or hypersensitivity reactions.)

To present in more detail some of these findings, the results of a recently completed study, done by this author in collaboration with Dr. Alan Woods and his co-workers at the Wilmer Institute of John Hopkins Hospital, are given in Table III It is to be noted that there is a significantly higher percentage of positive dye tests and skin tests in cases of granulomatous uveitis than in the controls Sixty-two percent of the former group showed dye test antibodies at a titer of 1 64 or higher, and 61% showed positive skin tests as compared with 20% and 30% respectively for the control group. In other studies, cases of non-granulomatous uveitis showed percentages very similar to persons without any ocular diseases, hence these cases are considered an adequate control

In this study, certain arbitrary criteria were established to make a presumptive diagnosis of ocular toxoplasmosis These were: 1.) a dye test titer of 1.64 or higher, and 2.) a positive skin test. When these standards were

rent toxoplasmic chorioretinitis. An experimental model of this chain of events has been described in the hamster by Frenkel (1954); it differs in that the hamster has high antibody levels at the time of development of ocular lesions.

As mentioned earlier, it is believed that the localization of parasites in the eye during the acute stage of acquired toxoplasmosis is fortuitous Obviously, it does not occur in every case. A comparison of the statistics on the incidence of toxoplasmosis and of chorioretinitis indicates it must occur relatively infrequently, just as symptomatic cases of lymphadenopathic toxoplasmosis must occur much less frequently than asymptomatic infections Nevertheless, because of its chronic and serious nature, it is believed that ocular toxoplasmosis may be the most important manifestation of this infection.

Support for this contention is necessarily based on statistical studies The reasons for this are as follows

1.) It is not feasible to attempt demonstration of the parasite in ocular tissues except in the event of enucleation or other surgery.

2) Because of the usually chronic nature of ocular toxoplasmosis serological tests on such cases may not show the rising antibody levels which are *characteristic of the acute systemic infection*

3.) Antibody levels in cases of ocular toxoplasmosis may be at the same titers as are frequently found in the general population. This has been amply demonstrated by the serological studies on Wilder's cases, some of whom showed very low levels of antibody only a few months after enucleation, and by the case of Jacobs, Fair and Bickerton. In the latter, the dye test showed *a stable titer of 1·64 prior to enucleation of the eye, the toxoplasmin* intradermal test was positive, and the complement fixation test remained negative. *Toxoplasma* was isolated from the ocular tissue following enucleation

Thus, in ocular disease, we are forced generally to rely on serological tests to attempt a diagnosis, and the serological data do not supply us with definitive information A possibility that under certain circumstances serial serolog*ical tests may be of aid in the diagnosis of suspected ocular toxoplasmosis* will be discussed further below. At the moment, however, from the standpoint of the estimating the frequency of the disease, the qualitative data of positive dye and skin tests must be relied on as indicating that *Toxoplasma* may be considered in the differential diagnosis of chorioretinitis or uveitis.

Exact figures on the frequency of ocular toxoplasmosis cannot be offered. However, comparative studies of the occurrence of positive serological or skin test reactions among groups of patients with granulomatous uveitis, as compared with control groups, have been performed in a number of countries. A tabulation of some of these investigations and their results is given in Table II. There is, in all the series listed, a definitely higher prevalence of positive

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TABLE III

Results of dye and skin tests in different types of uveitis

Type of uveitis	Total number of cases	No and incidence of positive dye tests	No and incidence of positive skin tests
Non-granulomatous	86	17 (20 %)	26 (30 %)
Granulomatous	107	67 (62 %)	65 (61 %)

used, and the cases were further analyzed as to presence or absence of evidence indicative of other possible etiologies to which the same type of clinical picture might presumably be due, the findings were as presented in Table IV. On the basis only of those cases for which no other etiology was discovered, at least 35 % of the cases of granulomatous uveitis appear to be possibly toxoplasmic in origin. If, in addition, it is assumed that some of the "uncertain" cases, in which similar evidence of other etiologies existed as well as evidence for toxoplasmosis, were indeed the latter, then the percentage considered toxoplasmic can soar to still higher levels. I would caution against too much enthusiasm in the acceptance of such an interpretation, because it is undoubtedly true that some of the presumed toxoplasmic cases are of different etiology. Nevertheless, the figures are impressive and indicate the importance of *Toxoplasma* in ocular disease.

It should be mentioned that, in this study, practically all the cases studied came from the Atlantic seaboard of the United States. The distribution, by age, in the granulomatous and control groups was also similar. It seems hardly likely that, in this study and those of others cited earlier, differences in age and locality could account for the generally higher reactions found among cases of granulomatous uveitis.

Statistically, therefore, it appears highly likely that toxoplasmosis is an important cause of uveitis. The diagnosis of the individual case, however, is at present a purely presumptive matter. Can further information on serological test reactions in such cases lend any more certainty to the diagnosis?

An analysis of dye test reactions in our series of cases reveals a somewhat

TABLE IV

Patients classified as to presence or absence of various possible etiologies of granulomatous uveitis

No of cases	Evidence for "conventional" etiologies	Positive for toxoplasmosis	Positive for toxoplasmosis and "conventional" etiologies	Undetermined
107	29 (27 %)	37 (35 %)	12 (11 %)	29 (27 %)

TABLE V

Intensity of positive dye tests in patients with presumed ocular toxoplasmosis

Condition of lesions	No. of cases	Less than 1 256	More than 1 256
Active	24	7 (29 1 %)	17 (71 9 %)
Inactive	13	6 (46 1 %)	7 (53 9 %)

higher percentage of high dye test titers among patients with active chorioretinal lesions than among chronic cases (Table V). While low antibody titers may be present in cases of ocular toxoplasmosis, nevertheless high antibody levels may be considered as more significant in certain instances I should like to point out that these judgments may be made only in those cases where the clinical appearance is consistent with what we know histopathologically about toxoplasmic uveitis; i. e. where the clinical appearance is that of a focal or disseminated chorioretinitis, or where the history indicates that such was the initial lesion. The clinical diagnosis of granulomatous uveitis, in cases where the fundus cannot be visualized, may be confused with the late manifestations of other ocular diseases such as tumors or subretinal hemorrhages (Coats' disease). Several such cases can be cited from the author's experience

One other phenomenon needs study in regard to its importance in the serological diagnosis of ocular toxoplasmosis. This is the fluctuation in antibody levels on serial dye tests. In the case studied by Jacobs, Fair, and Bickerton, there was rapid fluctuation in titer on successive tests following enucleation of the eye, in marked contrast to the low titers found prior to surgery (Table VI). Hogan, Thygeson, and Kimura (1952) and Hudson (1954) also have reported considerable variations in titer in patients presumed to have ocular toxoplasmosis. In a number of cases we have studied, such fluctuations in titer have been seen; in some instances the range in titer has been from 1:1024 to 1:16 or occasionally to 1:1 This fluctuation, so far as I have been able to evaluate the data, has no relation to skin testing, which can be followed by a fall rather than a rise in titer. Whether these changes are characteristic of ophthalmic cases, or are also found in "normal" individuals is not known. We are at present investigating this point, because

TABLE VI

Dye test titers obtained on serum of A J H prior to and after enucleation of his left eye on Jan 12, 1954, because of toxoplasmic chorioretinitis

	1953			1954					
Date	12/14	12/16	12/21	1/19	1/26	2/2	2/9	2/16	2/23
Titer	1 64	1 64	1 64	1·4,096	1 128	1·1,024	1 256	1 256	1 256

this basis must be established before varying titers can be evaluated in relation to ocular toxoplasmosis.

Experimental ocular toxoplasmosis and chemotherapy. Two mechanisms of pathogenesis of ocular lesions in toxoplasmosis have been postulated by Frenkel (1949). These are. 1.) Cell damage due to invasion by *Toxoplasma* and proliferation of the parasites within the cells, and 2.) an antigen-antibody reaction resulting from the rupture of pseudocysts in already sensitized tissue. There is evidence for both of these views; only the former concerns us here.

Wilder has found rosette-like forms of the parasite in necrotic retina, which appear to be indicative of proliferating parasites. Thus, there is a rationale for the use of chemotherapeutic agents in an attempt to control such proliferation in cases with active chorioretinal lesions

We have been carrying on studies on the production and treatment of anterior uveitis in rabbits on the basis of this rationale. We are able to produce anterior uveitis in rabbits by the injection into the anterior chamber of a relatively avirulent strain of *Toxoplasma*. Because this strain does not produce fatal infection, it allows us to study the ocular disease for a longer period than is possible with virulent strains. The first signs of uveitis appear in 1 to 4 days, depending on the size of the inoculum, and the eye is grossly inflamed in about 7 days The visible lesions are intense perilimbal injection, extensive edema and hyperemia of the iris, and an outpouring of exudate from the surface of the iris. The eye remains inflamed for about 2 or 3 weeks following the first appearance of symptoms, and then gradually subsides

We have tested pyrimethamine, sulfadiazine, Diasone [disodium (sulfonylbis (p.phenylenimino) dimethanesulfinate)], DDS (4;4'diamino-diphenylsulfone), all given orally; and have done a little work with 2 dihydrotriazines, given subcutaneously. The results of the studies with pyrimethamine and sulfadiazine indicate that, at levels of 100 mg/kg/day, starting on the day of infection, these drugs are able to prevent the development of severe uveitis in rabbits. (Table VII). The combination is more effective than either alone. The level of 100 mg/kg of pyrimethamine is surprisingly non-toxic to rabbits, considering the toxicity of this drug at dosages above 1.25 mg/kg in monkeys (Schmidt *et al*, 1953). The difference is probably due to rapid detoxification, elimination, or neutralization of the drug in the rabbit, and probably explains the high levels necessary to produce a therapeutic effect in this animal. The efficacy of the combination of sulfadiazine and pyrimethamine certainly warrants further trials of this regimen in humans. Thus far, some encouraging results have already been reported, (Ryan *et al*, 1954; Jacobs *et al*, 1956).

Diasone was found ineffective against ocular toxoplasmosis in rabbits. DDS, in confirmation of the results of Beverley *et al* (1954) appears at least

TABLE VII

Summary of treatment of experimental anterior uveitis of rabbits

Treatment	Number of rabbits	Total of grades of uveitis		Number of rabbits with uveitis of grade				
		O D	O S	0	1+	2+	3+	4+
Pyrimethamine .... 100 mg./kg.	6	8	0	2	2	1	1	
Sulfadiazine .... 100 mg./kg	6	11	3		3	2		1
Pyrimethamine and Sulfadiazine ... 20 and 40 mg./kg	6	9	2	1	2	2	1	
None	6	21	12				3	3

Inoculum: 113-CE 5000 O D, 500 O S

partially effective in preventing or reducing the severity of the experimental uveitis in these animals Similarly, the two dihydrotriazines [D-63.HCl – 4,6-diamino-1-(3',4'-dichlorophenyl)-2-ethyl-1, 2-dihydro-s-triazine, and D-110 HCl – 4,6-diamino-1-(*m*-chlorophenyl)-2-(*n*-hexyl)-1,2-dihydro-s-triazine] appear active in ocular toxoplasmosis of rabbits at dosages of 100 mg/kg This is consistent with the findings of Foley *et al* (unpublished) who are reporting the activity of these compounds against murine toxoplasmosis.

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Great Britain

OCULAR TOXOPLASMOSIS

J K.A. Beverley

Ocular abnormality is by far the most constant clinical manifestation of congenital toxoplasmosis (2, 3). Often it is the only one but it is not invariably present. One or both eyes may be affected and to differing extents. Most frequently the pathological changes in the eye are confined to the choroid and retina but sometimes there is extension to adjacent structures leading to vitreous haemorrhage, retinal detachment, anterior uveitis, anterior and posterior synechiae, cataracts or panophtalmitis and eventual microphthalmos.

Advice is sometimes sought at an early age by parents who have noticed obvious gross damage to the eye and in these cases it may not be possible to examine the fundus and see the typical choroido-retinitis. Some cases are brought later in their first year because of searching nystagmoid movements, a squint or an altered light reflex; usually these cases have a lesion involving the macular area. Others are brought at a still later date on account of suspected defective vision; many of these are referred to the school oculist by their teacher. Still others are found accidentally at the time of a first routine ophthalmoscopic examination; these patients usually have small lesions nearer the periphery.

The ophthalmoscopic appearances with which most of you will be familiar are those of inactive cases. (Fig 1.) The white patches are areas where the sclera is exposed as a result of necrosis of the retina and choroid with consequent loss of the pigment. Some pigment remains and is visible where it is not covered by retina It is usually more marked towards the edges of the lesion and occurs in irregular patches and streaks (1) Sometimes choroidal vessels are visible. (Fig. 2.) Section of a quiescent lesion resembles an ulcer with overhanging edges and a base of sclera. (Fig. 3.)

Much more rarely one may see an active case. Here the view of the fundus is obscured by vitreous haze but yellowish-white wool-like raised areas of the retina can be seen The retinal blood vessels traversing the area are empty and unseen. As the state becomes inactive, the vitreous clears, the retinal oedema subsides and through the necrotic avascular remnants of the choroid and retina the sclera becomes visible as white areas. Here and there remnants of pigment become apparent Fig. 4 shows in section a necrotic area in a subsiding case, while Fig 5 shows the gross oedema of the adjacent

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retina, principally in the layers superficial to the inner nuclear layer; this can be contrasted with a part of normal retina from the same eye. (Fig. 6.)

Sometimes the choroido-retinitis occurs before birth while in other cases it does not appear until a few weeks after birth (2). You all know the type of case which is apparently normal at birth and which develops gross changes a few weeks later. I do not think any of you will doubt that these are congenital infections even if only because the mother has high antibody levels. Why is there so often a delay between infection and the appearance of clinical signs? There are four phenomena which I would like to mention and which may help in considering this question. First, in sections of eyes with acute choroido-retinitis it is rare to find toxoplasms, terminal colonies, or cysts. Second, infiltration with eosinophile leucocytes and plasma cells is often marked. Third, necrosis occurs and is non-vascular in origin and therefore we should consider that hypersensitivity is a possible factor. Fourth, it is known that sensitisation of Rh +ve foetal red cells by passively acquired Rhesus antibody from an Rh −ve mother, leading to haemolytic disease, is commoner the greater the similarity between foetal and maternal red cells as far as the other blood group antigens are concerned. Could the same not apply in congenital toxoplasmosis thereby explaining why in some cases there is an ante-natal reaction, and why in others it is delayed until a few weeks after birth when the child is presumably developing its own antibodies to bring about sensitisation?

A clinical diagnosis should, if possible, be corroborated by a laboratory one. The ideal laboratory diagnosis is isolation of the parasite by inoculation of body fluids or tissue suspensions into susceptible animals In cases where eye changes are the only clinical sign, this is possible only when enucleation is performed for either intractable pain or for cosmetic reasons A histological diagnosis (10) is sometimes conclusive but more often it is very dubious. The most feasible laboratory tests are the dye test (9) and the complement fixation test. The latter, when using an antigen prepared from chick chorio-allantoic membranes by high speed centrifugation, is not as sensitive as, but is more specific than, the dye test. We have come to regard a positive C.F.T. to a titre of 1:8 or more as indicating either active or recent toxoplasma infection. From the diagnostic point of view, it is perhaps unfortunate that the complement fixing antibodies disappear early, often within two years. The dye test antibodies persist for much longer, but unfortunately their diagnostic importance is limited by the fact that many 'normal' people have dye test antibodies. The extent of these antibodies in 'normal' people and their concentration varies with the locality (4). A survey of dye test antibodies in townsfolk in England is shown in Table 1.

We, therefore, regard a titre of 1:32 as suspicious, 1:64 as probable and 1:128 as strong presumptive evidence of toxoplasmic aetiology. The fall in



Fig 1

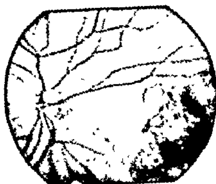


Fig 2

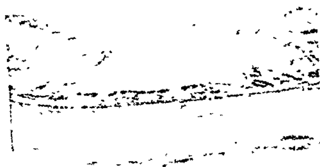


Fig 3



Fig 4

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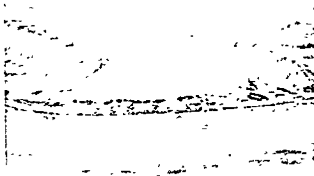


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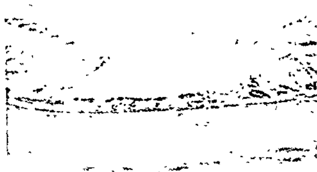
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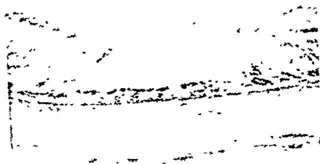
Sometimes the choroido-retinitis occurs before birth while in other cases it does not appear until a few weeks after birth (2). You all know the type of case which is apparently normal at birth and which develops gross changes a few weeks later. I do not think any of you will doubt that these are congenital infections even if only because the mother has high antibody levels. Why is there so often a delay between infection and the appearance of clinical signs? There are four phenomena which I would like to mention and which may help in considering this question. First, in sections of eyes with acute choroido-retinitis it is rare to find toxoplasms, terminal colonies, or cysts. Second, infiltration with eosinophile leucocytes and plasma cells is often marked Third, necrosis occurs and is non-vascular in origin and therefore we should consider that hypersensitivity is a possible factor. Fourth, it is known that sensitisation of Rh +ve foetal red cells by passively acquired Rhesus antibody from an Rh −ve mother, leading to haemolytic disease, is commoner the greater the similarity between foetal and maternal red cells as far as the other blood group antigens are concerned. Could the same not apply in congenital toxoplasmosis thereby explaining why in some cases there is an ante-natal reaction, and why in others it is delayed until a few weeks after birth when the child is presumably developing its own antibodies to bring about sensitisation?

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We, therefore, regard a titre of 1:32 as suspicious, 1:64 as probable and 1:128 as strong presumptive evidence of toxoplasmic aetiology. The fall in


Fig 1


Fig 2


Fig 3



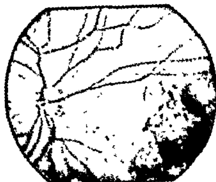
retina, principally in the layers superficial to the inner nuclear layer; this can be contrasted with a part of normal retina from the same eye. (Fig. 6.)

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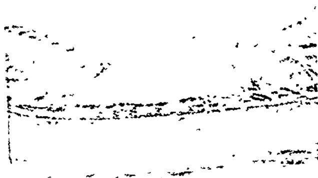

Fig 3


Fig 4

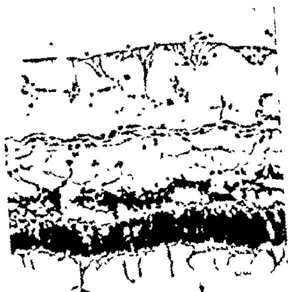


Fig 5



Fig 6



TABLE 1

Titre	Age Group					
	0-9	10-19	20-29	30-39	40-49	Over 50
1/4–1/6	1	6 8	25	23	19	27
1/16–1/32	0	2 7	2	1	3	1
1/32–1/64	1(?)	0	2	1	0	1
1/64	0	0	0	0	0	0
No. of cases	106	73	101	105	108	105

titre of dye test antibodies with the lapse of time since infection is illustrated in Table 2 which summarises the findings in 56 cases of presumed congenital toxoplasmic choroido-retinitis. It will be seen that the dye test is more likely to give a significant result the younger the patient.

TABLE 2

Dye Test Titres in Children with Choroido-retinitis

	No of cases	Average	Range
At birth	2	1/3,750	1/5,700–1/1,800
	4 mothers	1/7,640	1/12,500–1/1,050
Up to 1 yr	8	1/850	1/2000–1/100
1 yr to 5 yrs.	12	1/374	1/1000–1/46
6 yrs to 10 yrs	12	1/225	1/860–1/15
11 yrs to 15 yrs	11	1/51	1/160–1/10
16 yrs to 20 yrs.	11	1/30	1/98–1/10

There are many cases which, on clinical grounds, are suggestive of congenital toxoplasmosis and yet the patients have no demonstrable antibodies We have tested the mother's sera in about 60 such cases, and nearly all of them are positive. Table 3 shows the results in 10 cases with hydrocephalus and choroido-retinitis The low patient's titre in cases 2, 3, and 10 is probably the residue of passively acquired antibody. All the mothers, except one, had dye test antibody, four to a reasonably high titre. Case 10 was put out of sequence because we followed this one over 15 months, and from Table 4 we see the disappearance of passively acquired antibody by the fourth month, a suspicion of active immunity at 10 months, and a definitely significant antibody level at 15 months. We think that the infection was almost, but not quite, eliminated by the passively acquired antibody, and that later there was a mild recrudescence of infection accompanied by development of actively acquired antibody We presume that in other cases, when the infection is completely eliminated by the passively acquired antibody, the child does not develop active immunity.

TABLE 3

Cases with Hydrocephalus and Choroido-retinitis of doubtful aetiology

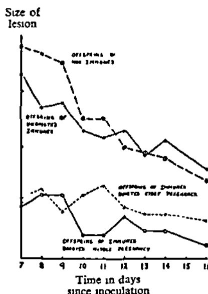
	Dye Test Titre		Age when test made
	Mother	Child	
1	1/11	–ve	6 weeks
2	1/100	1/4	7 weeks
3	1/16	1/4	4 months
4	1/8	–ve	6 months
5	1/50	<1/4	8 months
6	–ve	–ve	8 months
7	1/32	–ve	1½ years
8	1/75	–ve	4½ years
9	1/14	–ve	5 years
10	1/16	1/4	11 weeks

TABLE 4

Date	Age	Child	
		D T	C.F T
15. V.53	11 wks	1/4	–ve
15. VI 53	4 mths.	–ve	–ve
30.XII 53	10 mths.	1/4	–ve
20 V 54	15 mths	1/350	1/32

In an attempt to prove this theory it would be necessary to show that passively acquired antibody could neutralise toxoplasms. To this end we reared offspring from four groups of rabbits. In group 1, the mothers were non-immune (11 offspring); group 2 mothers were immunised 2–5 months before mating (16 offspring), group 3 were immunised 2–5 months before mating and boosted three days before mating (16 offspring) and group 4 mothers were immunised 2–5 months before mating and boosted 15 days after mating (12 offspring). When a month old, the offspring were inoculated just under the skin with a strain of toxoplasma non-fatal to rabbits. Graph 1 shows the sizes of the lesions in the four groups of offspring and suggest that passively acquired antibody does have a neutralising effect.

Remote complications of congenital choroido-retinitis do occur. Attacks of acute choroiditis superimposed on the old lesion are the commonest of these. In all probability the vast majority of so-called primary adult toxoplasmic choroido-retinitis cases are really re-activations of latent lesions (6, 8). Often there is a tell-tale patch of quiescent choroiditis in the other eye. Anterior uveitis may be of toxoplasmic aetiology but there is nearly always a pre-existing choroido-retinitis and the most likely explanation of



Graph 1

the anterior uveitis is that a sensitisation to posterior chamber break-down products has occurred. The association of stress in one form or another with these acute exacerbations is interesting. One of our cases had a fall and developed mild concussion 24 hours before a flare-up Another case, and one of the few who have developed a rise in antibody levels, had an exacerbation in each of two pregnancies The children were quite normal. Her antibody levels before, during and after an exacerbation are shown in Table 5.

With regard to treatment, if the condition is quiescent, no specific measures are indicated. On the other hand, if there is activity, specific treatment should be considered. Chemotherapy alone in either primary cases or in acute exacerbations of latent cases has given disappointing results Cortisone used in the relapse type of case does halt the acute response and presumably lessens extension of the damage, but subsequent further relapses do occur.

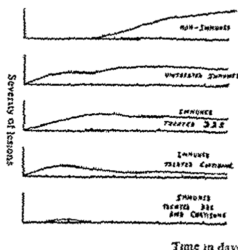
TABLE 5

Mrs O born 1922

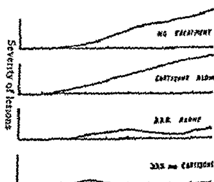
	Dye Test	C F T
1.IV.55	1/4	–ve
23.IV.56	1/550	–ve
11. V 56	1/1,600	1/80

Choroido-retinitis since 1941

Response of rabbits to inoculation of toxoplasms into the anterior chamber



Response of non-immune rabbits to inoculation of toxoplasms into the anterior chamber



Time in days since inoculation

Graph 2

Graph 3

We have not yet had an opportunity of trying a combination of chemotherapy and cortisone in humans but we have done preliminary experiments in rabbits.

Non-immune rabbits inoculated with living toxoplasms into the anterior chamber develop an anterior uveitis which progresses into a panophthalmitis and finally the animals die (Graph 2) If they are given 4,4' diamino-diphenyl-sulphone (D.D.S) alone, they recover but have an eye with considerable permanent damage Other animals given both cortisone and chemotherapy recover with a perfectly useful eye having had only minimal reaction in the acute stage.

Immune rabbits inoculated similarly and given no treatment develop a severe anterior uveitis. They recover but have permanent damage, as do rabbits given chemotherapy (Graph 3). Those animals given cortisone alone have less severe permanent sequelae while those given both chemotherapy and cortisone have only transient ill-effects.

If exacerbations of uveitis in humans are due to the activity of living toxoplasms, then treatment with a combination of chemotherapy and cortisone would be indicated We have a certain amount of evidence that toxoplasms multiply even in an animal possessing high titre antibody and capable of resisting a challenge dose, but this must not be taken to mean that all cases of recrudescence are caused by multiplying toxoplasms. Breakdown of dead pseudocysts liberating antigen, might possibly cause a local reaction. Antigen prepared from killed toxoplasms will induce a hypersensitivity reaction in immune humans (5) and it will produce an acute uveitis in immune rabbits.

SUMMARY

The clinical types of ocular toxoplasmosis are outlined. Attention is drawn to the occurrence of pathological lesions before birth in some cases and after birth in others. A mechanism is suggested which may account for these two kinds of reaction The significance of laboratory findings is discussed and their greater diagnostic value in younger patients is emphasised. The possibility of in utero neutralisation of toxoplasms by passively acquired antibodies is mentioned and a case passing through a negative phase is presented. Experimental work suggesting that passively acquired antibody can neutralise toxoplasms is described. Remote complications and their relation with stress are noted Treatment of humans and experimental animals is discussed and the synergic effect of chemotherapy and cortisone is shown.

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EPIDEMIOLOGICAL ASPECTS OF TOXOPLASMOSIS

Department of Preventive Medicine, State University of New York,
Upstate Medical Center at Syracuse, New York, USA

EPIDEMIOLOGICAL ASPECTS OF TOXOPLASMOSIS[1]

HARRY A. FELDMAN

One of the advantages of discussing a disease problem under the heading "Epidemiological" is that it gives the speaker full license to consider whatever aspects of the problem excite his fancy. There is much about toxoplasmosis, however, to excite all our fancies, whether we be pediatricians, internists, ophthalmologists, radiologists, serologists, parasitologists, or "shoe-leather" epidemiologists. It is in this vein that I have planned today's presentation It is my hope that there will be something of interest for each of you, even though you represent many diverse fields of interest. Lantern slides will be used liberally. It is my hope that this will facilitate the overcoming of whatever language inconveniences exist between us The data are drawn from our own experiences, which is not to deny that others may have reached similar or other conclusions on the basis of their experiments I am certain, though, that you will agree with me that all of this is only the beginning of our efforts to solve the problems posed by *Toxoplasma gondii*

Our data are based primarily upon the dye test. I shall not, at this time, enter into a discussion of the question of the specificity of this test which has been raised by some, except to say that we do not believe that significant cross-reactions have been demonstrated

Let me show you several general slides to orient those who may not be too familiar with the parasite and then we can proceed to our main subject This is *Toxoplasma gondii* as stained with Wright's stain in an impression smear of an infected mouse's brain. The principles of the dye test are illustrated next It is important to recognize that the dye plays no active role in this reaction; a positive or negative test can be recognized, readily, in the absence of the dye Although somewhat difficult to photograph well, the third slide illustrates stained und unstained parasites in a test mixture.

In the next two slides are tabulated the dye test titers by the intervals from birth of children with congenital toxoplasmosis and their mother's sera which were obtained at the same time (1). It is apparent that antibody may be demonstrable for many years, but that it persists at different levels in different

1. Supported by a series of grants from the National Institutes of Health, Bethesda, Maryland.

individuals. Whether it ever disappears cannot be answered by data such as these. The solution to that question requires that serial studies be conducted on the same individuals; we have been unable to accomplish this, as yet, but we have such studies in progress.

The differences in antibody patterns observed in congenital toxoplasmosis and with passive transfer antibody are illustrated next. You will note that by the third month (2) there has been a marked decrease in infant levels when the antibody has been passively transferred

TABLE I

Toxoplasma dye test antibody patterns illustrating passive transfer and congenital disease

Situation	Days post-partum	Dye test titers	
		Maternal	Infant
Passive transfer	0	256	128
	42	-	128
	90	-	16
	132	256	4
Congenital toxoplasmosis	50	1024	512
	127	1024	1024

Reprinted from (2).

With this background, we can proceed to several more detailed studies. We have conducted serological epidemiological surveys among 10 human populations (1) and illustrations of several of these follow Among 21 Alaskan Eskimos (10 of whom were in the age group 30–39 years) none was found to have dye test antibodies. Fifteen of 236 Navajo Indians who live in the Arizona desert had some antibody, but only 10 had titers of 1.16 or more. Notice that these are randomly distributed and not related to the ages of the donor. The same is true of the results obtained among 16 of 108 Icelanders, although here there is some suggestion that the younger individuals have the higher titers. This is more apparent in the titers observed in residents of Portland, Oregon, where 70 of 293 residents were found to have some antibody. The story was quite different, however, in Honduras and Tahiti, where most of the population yielded positive results. All 10 populations are summarized on the next slide. It is seen readily that there are marked variations in frequency, from place to place – even within a single country. This illustrates very well the hazards involved in generalizing from the serological results obtained in one area to questions of frequency and relation to possible reservoirs and clinical states in other locales.

Next, we compare the Navajo and Tahitian surveys. It is evident that if one were attempt to relate a given clinical syndrome to infection with toxo-

TABLE II

Frequency of toxoplasma antibodies among 10 "normal" human populations

Population	Number tested	Positive*	
		No	Per cent
Eskimo	21	0	0
Navajo Indian	236	10	4
Iceland	108	12	11
Portland, Ore.	293	51	17
St. Louis, Mo.	184	47	26
New Orleans, La	270	84	31
Pittsburgh, Pa.	144	51	35
Haiti	104	37	36
Honduras	266	170	64
Tahiti	121	82	68
Totals...	1.747	544	31

*Dye test titer 1 in 16 or more.

Reprinted from (1)

TABLE III

Comparative incidence of antibodies for toxoplasma (dye test) among Navajo indians and Tahitians

Age group	Navajo			Tahiti		
	Tested	Positive*		Tested	Positive*	
		No	%		No	%
0- 4	5	0	0	20	3	15
5- 9	15	0	0	20	15	75
10-19	37	2	5	20	18	90
20-29	40	0	0	20	14	70
30-39	30	2	7	22	17	77
40-49	37	2	5	8	6	75
50+	40	4	10	11	9	82
Totals	204	10	5	121	82	68

plasma in these two areas, he would have different degrees of success. Almost any disease state that would be studied in Tahiti would be found to have antibodies for toxoplasma to a significant degree but a positive conclusion, however, might well be incorrect

In the following slide, I have taken the liberty to compare our Navajo data

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5–9	15	0	0	20	15	75
10–19	37	2	5	20	18	90
20–29	40	0	0	20	14	70
30–39	30	2	7	22	17	77
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In the following slide, I have taken the liberty to compare our Navajo data

with the Sheffield survey of Beverley and associates. The similarity of the two is striking. In view of the marked climatic, economic, ethnic and housing differences between these two populations this result is most unexpected and I have no explanation for it.

Among 1,191 people, we found both males and females to have antibodies to the same extent (1). Similar observations have been reported previously by us and others, as well. *We have not encountered any evidence that suggests* that human to human transfer occurs often, if at all.

We, also, have had an opportunity to examine the sera of 24 different animal populations. The following three slides illustrate some of the findings in them You will note that the same variations in frequency which were detected among humans, also obtain among animals Generalizations thus are dangerous in both situations.

TABLE IV

*Toxoplasma dye test antibody titers observed in 5 cattle herds**

Herd	Source	No. tested	Positive**	
			No	Per cent
1	Ithaca, N Y	10	0	0
2	Central N Y	66	0	0
3	Ithaca, N Y	24	9	38
4	Ithaca, N Y.	33	16	49
5	Navajo (Ariz)	23	0	0
	Totals .	156	25	16

* Reprinted from (1)
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TABLE V

*Frequency with which various manifestations of congenital Toxoplasmosis were reported in 180 cases**

Manifestation	Total cases	Reported	
		No	%
Chorioretinitis	150	141	94
Cerebral Calcification	158	93	59
Psycho-Motor Retardation	142	64	45
Convulsions	131	51	39
Microphthalmia	135	48	36
Hydrocephaly	147	32	22
Microcephaly	150	31	21

* Studied by the author.

TABLE VI

Age distribution of mothers of infants with congenital toxoplasmosis

Age group (Years)	Number	Per cent
18–29	72	73 5
30–39	26	26 5
Totals ..	98	100

Now let us turn to two important human toxoplasma problems – congenital toxoplasmosis and acquired eye disease Infants with the former may be born in all months of the year, there appears to be no seasonal factor in the acquisition of the parasite. Some of the clinical findings in the cases of the congenital form that we have studied are tabulated in the next slide. You will note that almost no child escapes some injury, that almost all have eye disease and half have cerebral calcifications and mental retardation. On the other hand, in studies of children with cerebral palsy or blindness or behavior problems, we have been unable to detect antibodies for toxoplasma to any significant degree

Does all chorioretinitis in children mean congenital toxoplasmosis? Not in our experience Even in the first year of life, only half of the cases could be related to this cause The same was true, also, of cerebral calcification. These data should not be interpreted as being applicable to other areas in view of what I have previously demonstrated. We are in desperate need of additional such studies but they will have little meaning unless they are conducted in relation to the "normal" population of limited geographic areas.

The age distribution of mothers of congenitally affected children is summarized next. They tend to be the younger mothers which is quite different from the problem of mongolism, for example, and might support the thesis that the mother has to be susceptible to infection in order to have her fetus infected

Congenital toxoplasmosis does produce pre-maturity but as is illustrated next, is, perhaps, not an outstanding cause of this complication of pregnancy.

Does the congenital disease recur in the obstetrical experience of the same mother? We have not seen this nor do other fetal hazards appear to be induced by one such event in the reproductive life of a given mother.

Lastly, we come to the vexing problem of chorioretinitis Among congenital cases, involvement of both eyes is the rule This speaks for bloodstream dissemination of the offending organism as the mechanism for producing the

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30–39	26	26 5
Totals .	98	100

Now let us turn to two important human toxoplasma problems – congenital toxoplasmosis and acquired eye disease. Infants with the former may be born in all months of the year, there appears to be no seasonal factor in the acquisition of the parasite Some of the clinical findings in the cases of the congenital form that we have studied are tabulated in the next slide. You will note that almost no child escapes some injury, that almost all have eye disease and half have cerebral calcifications and mental retardation. On the other hand, in studies of children with cerebral palsy or blindness or behavior problems, we have been unable to detect antibodies for toxoplasma to any significant degree.

Does all chorioretinitis in children mean congenital toxoplasmosis? Not in our experience Even in the first year of life, only half of the cases could be related to this cause. The same was true, also, of cerebral calcification These data should not be interpreted as being applicable to other areas in view of what I have previously demonstrated. We are in desperate need of additional such studies but they will have little meaning unless they are conducted in relation to the "normal" population of limited geographic areas

The age distribution of mothers of congenitally affected children is summarized next. They tend to be the younger mothers which is quite different from the problem of mongolism, for example, and might support the thesis that the mother has to be susceptible to infection in order to have her fetus infected.

Congenital toxoplasmosis does produce pre-maturity but as is illustrated next, is, perhaps, not an outstanding cause of this complication of pregnancy.

Does the congenital disease recur in the obstetrical experience of the same mother? We have not seen this nor do other fetal hazards appear to be induced by one such event in the reproductive life of a given mother.

Lastly, we come to the vexing problem of chorioretinitis. Among congenital cases, involvement of both eyes is the rule This speaks for bloodstream dissemination of the offending organism as the mechanism for producing the

with the Sheffield survey of Beverley and associates. The simil[illegible] is striking. In view of the mark[illegible] diff[illegible] [illegible] people, we found both males and females to have antibodies to the same extent (1) Similar observations have been reported previously by us and others, as well. We have not encountered any evidence that suggests that human to human transfer occurs often, if at all.

We, also, have had an opportunity to examine the sera of 24 different animal populations. The following three slides illustrate some of the findings in them You will note that the same variations in frequency which were detected among humans, also obtain among animals. Generalizations thus are dangerous in both situations.

TABLE IV

*Toxoplasma dye test antibody titers observed in 5 cattle herds**

Herd	Source	No tested	Positive**	
			No	Per cent
1	Ithaca, N Y	10	0	0
2	Central N Y	66	0	0
3	Ithaca, N Y.	24	9	38
4	Ithaca, N Y.	33	16	49
5	Navajo (Ariz.)	23	0	0
	Totals .	156	25	16

* Reprinted from (1)
** Titer 1.16 or more

TABLE V

*Frequency with which various manifestations of congenital Toxoplasmosis were reported in 180 cases**

Manifestation	Total cases	Reported	
		No	%
Chorioretinitis	150	141	94
Cerebral Calcification	158	93	59
Psycho-Motor Retardation	142	64	45
Convulsions	131	51	39
Microphthalmia	135	48	36
Hydrocephaly	147	32	22
Microcephaly	150	31	21

* Studied by the author.

Department of Bacteriology, University of Sheffield,
Great Britain

THE EPIDEMIOLOGY OF TOXOPLASMOSIS

C. P. Beattie

In spite of the large amount of work that has been done, especially in the last ten years, much remains to be discovered about the epidemiology of toxoplasmosis.

It is known that the parasite has a wide, perhaps world wide, geographical distribution and infects many species of birds and mammals, including man. Many observations in animals and a few in man show that it may persist in the tissues for long periods without causing obvious illness. Indeed it is probable that infection is frequent, disease exceptional.

Infection causes the production of antibodies which can be detected by the Sabin-Feldman dye test, the complement fixation test and the skin test. These antibodies are quite commonly found in the population of many countries and increase in frequency from childhood to adult life.

On the basis of dye test and skin test results it has been estimated that from one quarter to three quarters, or even more, of the populations of various countries are, or have been, infected. Positive complement fixation test results are less frequent (1½ to 20 %), but there are such great differences in technique that comparison is impossible Of particular interest is Feldman's (1952) finding that over three quarters of the population of Tahiti had dye test antibodies to a titre of 1.16 by the early age of nine. By contrast they were found in only 5 % of Navajo Indians, in no Eskimos and in 11 % of Icelanders.

Confidence that a high prevalence of toxoplasma dye test antibodies indicates a high rate of infection has, however, been disturbed by statements that in animals they may be produced by other agents. Muhlpfordt (1951) and Awad and Lainson (1954) found them in sarcocystis infection Moscovici (1954) found the contrary. Westphal (1952) found them in animals infected with trypanosomes, but Gronroos and Salminen (1955) found no correlation between toxoplasma antibodies and the presence of *Trypanosoma lewisi* in the blood of rats. Nor did Cathie (1957) find toxoplasma antibodies to significant titre in the serum of man with trypanosomiasis. Michalzick (1953) considered trichomonas infection a cause of confusion, but Cathie (1955) thought dye test antibodies to be no commoner in patients with trichomoniasis than in the general public.

broad effects of the congenital disease. Among acquired eye cases of unknown cause, uni-lateral involvement has been the rule. In our experience (which is admittedly not adequate since the data are not collected from one place) 60% of 399 cases of uveitis had no antibodies at all. When tabulated by ages there is a gradual increase of positives with age with an interesting and curious upswing in the age group 20–29 years. When the positive eye cases are tabulated by dye test titers opposed to their ages, the serological data are of suggestive importance in about 10% of the positives which represents approximately 5% of the sample. We have a study under way in our own community in which all eye cases are being studied by the same serological techniques which are being applied to the "normal" population of the same area. It is anticipated that by this method some light will be cast upon the important problem of the relationship between toxoplasmosis and acquired eye disease. I believe that this is the only epidemiological method that offers substantial help in gaining additional insight into this problem.

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For the time being, however, these results must be accepted with caution, tempered by the knowledge that nothing else has yet been shown to give the very high titres found in proved cases of active toxoplasmosis. It may be necessary to adopt an arbitrary titre and neglect results below that, but toxoplasma has been isolated in the presence of comparatively low titres. Antibodies, moreover, rise and fall. These are some of the difficulties in the way of using serological tests for epidemiological surveys.

Even were the extreme step to be taken of neglecting the results of serological surveys, evidence would still remain that toxoplasma infection is not infrequent. It is known that in numerous species of animals inoculation of toxoplasma usually leads to latent infection, disease being exceptional. It would be strange if this were not the case in man Toxoplasma has been isolated (Cole et al , 1953) or found on histological examination (Tomlinson, 1945; Mantz et al., 1949) in human beings who have shown no sign of active toxoplasmosis. Most importantly a history of illness is rarely given by mothers who give birth to babies with congenital toxoplasmosis

The question arises of why infection sometimes causes disease.

Two factors must be considered.– the parasite and the host.

There is little doubt that strains of toxoplasma vary in virulence (Erichsen and Harboe, 1953; Frenkel, 1954). Lainson (1955) found strains isolated from naturally infected, and apparently healthy, rabbits to be of low virulence and this has been confirmed in my laboratory. Some of the reported differences arise from the comparison of a naturally occurring strain with one that has been passed repeatedly through animals in the laboratory This is not the whole story, for, in common with others, we have found that some strains from man can be readily isolated by animal passage, others with difficulty. Nor is it just a matter of numbers. We have inoculated gland material from two patients into mice and, in spite of numerous passages over a period of months, only once in each case found a few toxoplasms When we later made passage into cortisone treated mice a high proportion of them died in a few days. Toxoplasms were numerous, yet when further passage was made into mice that had not received cortisone they remained well.

Resistance of the host undoubtedly plays a great part. The foetus is particularly susceptible, but even here there seems to be variation in susceptibility The frequence *with which toxoplasmosis has been reported* in twins seems greater than can be explained by the distribution of twins and just as at birth one twin is often the stronger, so one may show advanced signs of toxoplasmosis and the other, although infected, little or none. (Farquhar, 1950, Otto, 1953). In later life it has been found in association with another disease – malaria (Guimaraes, 1943), bartonellosis (Pinkerton and Weinman, 1940), tuberculous meningitis (Paul, 1954), reticulosarcoma (Finckh, 1954), whooping cough (Prior et al , 1953). The 16 dogs in which Campbell (1955)

Positive toxoplasma complement fixation reactions have also come under suspicion on the report of their being frequently found in tuberculosis (Hein, 1952), typhoid and paratyphoid (Scholta, 1954; Kulasiri, 1954) and malaria (Vermeil, 1953). In all these instances peritoneal exudate antigen was used. Cathie (1955) using an egg antigen, did not find complement fixation in 49 sera positive in the Widal reaction.

In my laboratory sera have been examined from persons suffering from, or who had suffered from, some of these diseases with the results shown in Table 1.

TABLE 1

Dye test and complement fixation test results in or after disease

Titres	Trichomoniasis		Trypanosomiasis	Malaria in the past.	
	D T	C F.T	D T	D T	C.F T.
<1/4	21	36	11	55	78
≥1/4	5	0	2	8	6
≥1/8	4	0	2	9	0
≥1/16	5	1	0	5	0
≥1/32	1	1	2	8	0
≥1/64	2	0	0	0	0
A C					1
Total	38	38	17	85	85

A greater frequency of high titre antibodies would be expected if these diseases stimulated the production of antibodies reacting with toxoplasma antigens.

The malaria sera were from persons who had malaria in the past, mostly in the last war. One is so free from antibody that it has been used as accessory factor in the dye test Serum was obtained from three patients before they were subjected to malaria therapy and one month later. In one case, no antibodies, and in the other two, low titre antibodies, which did not rise, were found before and after infection (Beverley, 1957).

Cathie and Cecil (1957) did not find a common antigenic factor in sarcocystis and toxoplasma. Human infections with sarcocystis are believed to be rare. Awad and Lainson (1954), however, examined serum from one human patient and my laboratory examined serum from another. In both cases the dye and complement fixation test for toxoplasmosis were negative.

Before the validity of positive serological reactions as indicators of toxoplasma infection can be properly assessed more work will have to be done. It is not unlikely that, perhaps with improvement in the tests or the introduction of new tests, confusion may not prove so great as has been feared.

For the time being, however, these results must be accepted with caution, tempered by the knowledge that nothing else has yet been shown to give the very high titres found in proved cases of active toxoplasmosis. It may be necessary to adopt an arbitrary titre and neglect results below that; but toxoplasma has been isolated in the presence of comparatively low titres. Antibodies, moreover, rise and fall. These are some of the difficulties in the way of using serological tests for epidemiological surveys

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found toxoplasma all showed histological changes suggestive of concurrent distemper.

The great unsolved problems are whence infection comes and how it is spread.

As far as man is concerned the only known way, apart from laboratory infections, is congenital. But the mother must acquire her infection somehow and at some time. Most commonly it is believed that, to infect her foetus, this must be during pregnancy. The possibility should not, however, be dismissed of foetal infection due to chronic latent maternal toxoplasmosis.

Evidence for this from animal experimentation is contradictory. Eichenwald (1948) failed to demonstrate it in mice, but Hellbrügge, Dahme and Hellbrugge (1953) and Wildfuhr (1954) showed it to be possible in rats, and Beverley (1959) showed not only that it may occur in subsequent pregnancies in mice, but also in successive generations. It does not, of course, follow that what happens in mice will happen in other animals. There are, moreover, many instances of active infection in older children and in adults which can not be accounted for by congenital transmission.

Man's domestic animals, particularly dogs and cats, have been suspected as a source Toxoplasmosis has often been described in these animals and actively infected dogs sometimes excrete toxoplasms in their urine, faeces and saliva. (For review see Jacobs, 1953). It is, therefore, not surprising that toxoplasmosis has been found in persons who have been in intimate contact with dogs suffering from toxoplasmosis. (Westphal and Finke, 1950); Fankhauser, 1951; Cole et al., 1953). Much more frequently, however, no history of intimate contact with a sick dog or cat is obtained and, particularly in view of the difficulty experienced in spreading infection amongst dogs (Jacobs et al., 1955) it is unlikely that casual contact would be enough.

Infection has been found in cattle, sheep, goats, pigs, rabbits and hares, but there is no definite proof of infection having spread from them to man. All that can be said is that we have found dye test antibodies more frequently

TABLE 2

Percentage of occupational groups – dye tests

Titres	General population	Abbatoir workers	Veterinary surgeons	Rabbit handlers	Rabbit trappers
≥1/16	2	12	12	43	67
≥1/32	1	3	4	25	42
≥1/64	0	1	2	12	21
Totals at risk	229	146	50	142	24

and to higher titres in veterinary surgeons and abattoir workers than in the general public. Still more striking is the frequency with which they are found in rabbit handlers and trappers. De Roever-Bonnett (1957), however, in Amsterdam found no increased incidence of toxoplasma antibodies in slaughter house workers.

These must, however, be regarded as special occupational risks except in so far as it may be possible for the consumption of infected meat to spread infection This will be considered later.

Infection in rats is well known. (Perrin et al., 1943; Eyles, 1952). Just as salmonella infection of man is spread by contamination of food by rat faeces, so might toxoplasmosis; but as the toxoplasms when excreted will be exposed to drying, to which they are not very resistant, and, unlike salmonella, are unable to multiply in dead material, this seems unlikely. More probably rats are a source of infection for dogs, cats, pigs and other animals that eat them.

Although toxoplasma infection has been reported in many species of birds it does not seem likely that infection could be transmitted to man from this source, unless by close contact, as suggested by Feldman and Sabin (1949) on the basis of serological results in five park keepers who handled pigeons.

The one animal rarely considered, yet the animal with which man is in closest contact, is man himself. There is as yet no definite evidence of man to man transmission, but it is suggestive that on several occasions more than one case of toxoplasmosis has been found in the same family. (Siim, 1951; Jacobsson, 1953). My colleagues and I, on the basis of the clinical picture of toxoplasmic lymphadenopathy accompanied by a dye test titre of 1:820 and a complement fixation titre of 1·8 rising to 1.16 diagnosed toxoplasmosis in a boy of 11. Subsequent enquiry revealed that four months later a younger brother aged 8 developed enlarged glands in the groin and later in the neck. When his serum was tested six months after the onset of illness it gave a dye test titre of 1.260 and a complement fixation titre of 1.5. Infection might have come from a common source and a dog which had been destroyed because of "distemper" was suspected, but none of the other eight members of the household, all of whom had as much contact with the dog as the two patients, gave dye test titres of over 1 10. The two boys slept in the same bed.

So it seems that the possible sources of toxoplasma infection are many. There remains the important question of how it is transmitted.

The usual modes of spread of infection must be considered·– (a) ingestion, (b) inhalation, (c) contagion, (d) transmission by insects.

From the superficial resemblance of toxoplasma to leishmania and trypanosoma and from the parasitaemia produced by infection, the last would seem an attractive possibility. It has, indeed, proved possible to infect a variety of insects by allowing them to suck the blood of toxoplasma infected

animals, but so far, with the possible exceptions reported by Woke and his colleagues (1953), it has not been possible to transmit infection by their bite.

Contagion is suggested by laboratory accidents in which infection has followed a needle prick and by the development of high titre antibodies after the bite of an infected rabbit. (Sabin et al., 1952).

Toxoplasms, perhaps from the saliva of an infected dog, perhaps from a *infected human being, might pass through scratches in the skin or external* mucous membranes.

Ingestion has been often considered. Successes and failures have resulted from attempts to infect animals in this way. The successes, for the most part, have followed on the ingestion of infected tissues, perhaps because the pseudocysts found in these tissues are more resistant to the action of gastric juice than are free forms. It is known that they can survive for a considerable time in animal tissues kept at refrigerator temperatures. Weinman and Chandler (1954) quote survival times of up to 30 days. On this basis and on the observation that epizootics of toxoplasmosis may occur in pigs and that these animals can acquire infection by eating infected mouse and rat tissue, these authors particularly suspect pork. In further support of this they point out that from 1 in 6 to 1 in 3 of the adult population of the United States has been infected with another parasite of the pig – trichina. Trichinosis is nothing like so common in England, but epidemics occasionally occur. When they do many more women than men are infected because of their habit of having a nibble at the meat before it is cooked. If toxoplasma infection were spread by pork it would be expected that toxoplasma antibodies would be found more frequently in women than in men. In England they are found equally commonly in men and women

In this connexion it is of interest that Rawal (1959) found practically no difference between the incidence of toxoplasma dye test antibodies in Hindu vegetarians and Moslem meat-eaters in Bombay. At a titre of 1.64 or more the percentages were 2 for the former and 1 for the latter. The numbers examined were 141 and 246.

It is easier to infect animals by nasal instillation than by feeding. The possibility of natural spread by the respiratory route is suggested by Pinkerton and Henderson's (1941) finding of toxoplasma laden macrophages in the alveoli of two adult patients, by Sim's (1951) report of catarrhal symptoms in toxoplasmic lymphadenopathy and by Cathie's (1954) isolation of the parasite from the saliva of a child. Rhinitis, moreover, has been described in infected animals and Kåss (1954) has found toxoplasms in the nasal secretions of infected rabbits.

Unfortunately for the theory of respiratory spread no one has yet succeeded in showing natural transmission from animal to animal by this route, although Kunert and Schmidtke (1954), by the highly artificial and drastic method of

exposing them to a toxoplasma containing spray, did succeed in infecting 8 of 9 guinea pigs but no mice.

Salivary contamination still seems a possibility, but perhaps entry of the parasite into the body is through the mucous membranes of the mouth and tonsils rather than through the lungs, stomach or intestine. The success achieved by nasal instillation might be explained by some of the infected liquid material having reached the tonsillo-pharyngeal region.

These then are some of the possible modes of infection. It would, however, be no surprise if toxoplasms were eventually shown to be transmitted by several routes or by some way not yet imagined

SUMMARY

Toxoplasmosis is of wide geographical and zoological distribution

Serological surveys indicate that from one quarter to three quarters of the adult population of various countries have been infected.

The specificity of serological tests has been questioned and defended.

There is other evidence that toxoplasma infection is not infrequent and generally latent.

The question arises of why infection sometimes gives rise to disease. Two factors are concerned – increased virulence of the parasite and decreased resistance of the host.

Man's domestic animals and man himself are potential sources of infection

Transmission by insects seem unlikely.

Possible vehicles of spread are – faeces, urine, saliva, nasal secretions and infected meat.

Possible routes of entry are through breaches in skin or external mucous membrane, through the mouth or through the nose

Infection by mouth can be produced in laboratory animals. The possibility must therefore be considered of infection by eating uncooked infected meat.

Nasal instillation readily infects some animals, but so far no definite evidence has been produced of natural infection by inhalation

It may be that the main portal of entry is through the tonsillo-pharyngeal region, which would be reached both by nasal instillation and through the mouth.

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Department of Microbiology, Yale University, New Haven, Connecticut, U.S.A

SUMMARY OF STUDIES ON THE EPIDEMIOLOGY OF TOXOPLASMOSIS WITH PARTICULAR REFERENCE TO THE ROLE OF SWINE[1]

DAVID WEINMAN and A. H. CHANDLER

The introduction about a dozen years ago of practical methods for mass testing for toxoplasmosis made population surveys possible. These surveys suggested that *Toxoplasma* infection was widespread in the normal adult population, infection rates running between 20 and 60%, according to the age group investigated, and the test and criteria employed. Such figures contrast sharply with the relatively few cases which receive clinical attention or post-mortem verification. Thus there is a marked discrepancy between the disease rate and the infection rate, which has been disturbing to those who consider *Toxoplasma* infection a rarity. This discrepancy results in part from our failure to recognize the infection because of atypical or undescribed symptoms, and in part from the fact that the greatest number of *Toxoplasma* infections are either asymptomatic or pauci-symptomatic from the onset, and it is only the exceptional individual who becomes ill.

If *Toxoplasma* infection is widespread in the population then there must be some common mechanism of transmission. Since it is known that animals, both wild and domestic, harbor *Toxoplasma*, current thought has assumed that the infection is conveyed from animals to man in some unidentified manner – perhaps by the intermediary of arthropoda, or by droplet infection, or the handling of infected animals or carcasses, or through the soiling of food by excreta, particularly of dogs, or finally, by the ingestion of contaminated food (6).

Our own investigations have explored the possibility that toxoplasmosis may be contracted through the eating of undercooked pork and that the swine themselves become infected by the oral route. Some of our results have already been reported elsewhere (7) so that I will summarize our findings as follows.

First, we have found that under experimental conditions pigs may be read-

1. These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Yale University, NR-130-201.

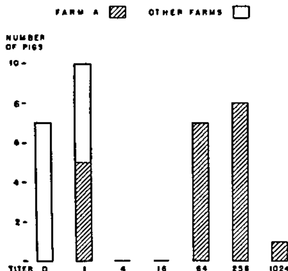


Fig 1

ily infected by feeding, either on infected rodents or by eating pork which contains *Toxoplasma* (8) Pigs infected orally were very rarely ill. They were however subject to a long-lasting infection, our evidence being that *Toxoplasma* may persist in a healthy-appearing pig for at least one year. Furthermore, the toxoplasmata which were recovered from these carrier pigs proved to be infectious and produced fatal infections when injected into test animals.

Second, we have found that pigs raised on farms give evidence of being infected with *Toxoplasma*. This is based on dye test results and as high as 40 to 50% of hogs from certain farms gave dye test titers which are considered to be at a significant level of 1.64 or more (Fig 1) These figures vary from farm to farm and are based on garbage-fed swine. We have recently been able to obtain additional material and we now know that these observations apply to farms in at least two areas in the eastern United States Connecticut and New Jersey. On at least one of these farms, rats, which ran wild among the pigs, have proved to be infected with *Toxoplasma* to the extent of 24% of the total number examined.

For our third point, we were able to show that humans who eat under-cooked pork are much more likely to have high titered toxoplasmic sera than is the normal population To demonstrate this, we took advantage of a biological marker which pointed out for us those individuals who ate under-cooked pork. That is, use was made of the high rate of trichinosis infection in the United States. Since trichinosis is almost always contracted from pork, individ-

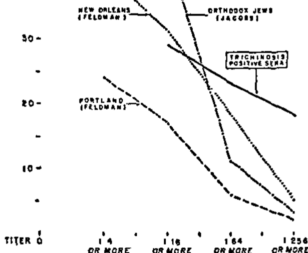


Fig. 2

uals suffering from trichinosis constituted just such a group known to have eaten underdone pork Following this lead, we found that persons with trichinosis titered 1 256 or more in the toxoplasma dye test in 18% of the cases, whereas in the normal population only 5% would be expected to titer 1:256 or more. In other words the trichinosis group gave approximately three times as many high-titered sera as did the normal group (Fig. 2).

Fourth, pork is offered for sale approximately four days after slaughter, and during this period is refrigerated slightly above the freezing point. Under such circumstances toxoplasmata remain alive for 10 to 30 days, that is, the survival period exceeds the minimum required, so that we might expect that infected pork would contain living *Toxoplasma* when the meat arrived in the kitchen.

Fifth, *Toxoplasma* which are contained in meat fragments show very considerable resistance to gastric digestion, and fragments of meat weighing less than two grams will survive the period of four hours of exposure which is the usual stay of meat in the stomach of man.

These figures apply to the ordinary "unencysted" form of *Toxoplasma*. When *Toxoplasma* occurs in pseudocysts the resistance to gastric digestion is approximately doubled This point has importance, for animals with chronic infections develop pseudocysts and pigs develop chronic infections, so that it appears that infected swine would provide toxoplasmata almost maximally resistant to the effect of gastric digestion.

Finally, *Toxoplasma* is infective when taken in by the oral route. We have found this to be the case for rodents, for pigs, and for *Macaca mulatta* monkeys, and our results parallel those obtained by others It is impossible to test the infectivity of *Toxoplasma per os* in man at present, since modern therapy is not uniformly successful, but we believe that all available evidence points to the susceptibility of man to infection by the oral route. In fact, at present the burden of proof would be upon him who maintained that man is so different from other animals as not to infect himself by feeding.

From this evidence the conclusion seems inescapable that in certain areas of the United States at least, pigs are a source of *Toxoplasma* infection for man and that inadequate cooking of infected pork provides a means whereby man becomes infected.

This is as much material as we have ready to report at present In a disease as widespread in as many different kinds of vertebrates as is toxoplasmosis there is probably more than one kind of transmission mechanism. And it may well be that man contracts the infection in more than one way. Certainly the fact that persons who do not eat pork may include some with high dye test titers seems to indicate this (Fig. 2). If so, then the relative importance of the pig-man cycle remains to be assessed. But we do feel that we have accumulated very suggestive evidence incriminating one definite specific transmitting mechanism. This may even prove to be one important means whereby toxoplasmosis is transmitted.

I should like to conclude this paper with an observation on the validity of the Sabin-Feldman dye test. This is one of the most widely used serological diagnostic tests for toxoplasmosis and the test used by us in the research now being reported to you.

A little over a year ago, it was reported that rabbits injected with *Trichomonas vaginalis* reacted positively in the *Toxoplasma* dye test, i e , the animals gave false positive reactions (1) From this, and from some data in the literature, the conclusion was drawn that the test might also be unreliable in cases of human *Trichomonas* infection If this were true, a considerable degree of uncertainty would be introduced into all investigations where the dye test was utilized, since *Trichomonas vaginalis* is a common human parasite.

In order to evaluate this criticism, a base-line of dye test titers in normal healthy blood donors from one locality was first established. About 100 sera were tested, and of these approximately 19 % titered 1 64 or above. Subsequently, sera were obtained from 24 women living in the same area and in the same age group who were infected with *Trichomonas vaginalis*, and very similar titers were obtained, i.e. 25% at 1:64 or more. These small differences between the two groups are not considered to be significant, and although our investigations are not yet complete, they have given us thus far no concrete evidence that *Trichomonas vaginalis* causes false positive dye tests.

Our investigations have taken us approximately four years and during this period confirmatory evidence has been published by others dealing with natural infections in pigs. A spontaneous epidemic in swine was reported in the United States (2) and from the same area it was subsequently shown that female pigs which were apparently in perfect health may transmit the infection to their offspring (5).

Of particular interest is the finding in Europe of infected swine. *Toxoplasma* has now been recovered from pigs in Denmark (4) and in Germany (3), and it may be asked whether swine infection is not widespread. If so, does pork serve as a source of human infection not only in the United States but also in Europe and elsewhere?

The much greater prevalence of trichinosis in the United States than in other countries indicates certain special epidemiological circumstances which makes one hesitant to draw conclusions as to the role of swine in the transmission of toxoplasma to man in Europe. However, now at least we do know that toxoplasmosis does occur in swine in Europe. The crucial point may be whether European housewives invariably cook their meat more thoroughly than we do in the United States. If so, then the risk from infected pork may be negligible, but *a priori* this does not seem likely. I would like to draw the attention of the members of the Congress to the solution of this question which is of the highest practical interest.

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LABORATORY DIAGNOSIS OF TOXOPLASMOSIS

Paediatric Clinic, University of Vienna, Austria

DIFFICULT AND UNSOLVED PROBLEMS IN THE DIAGNOSIS OF TOXOPLASMOSIS

O. Thalhammer

Not the technical aspects of the tests used in the diagnosis of toxoplasmosis, but four problems connected with their interpretation will be dealt with here. These are. (1) diagnosis of uncharacteristic cerebral defects on the basis of congenital toxoplasmosis, (2) early diagnosis in cases of acute acquired toxoplasmosis, (3) diagnosis of toxoplasmosis in the general laboratory independent of a living strain of toxoplasma, and (4) diagnosis of acquired chorioretinitis.

The first problem has three aspects. Firstly, congenital cerebral defects generally become recognizable the later in life the milder they are. Only severe cases are diagnosed already in infancy. The antibody titres commence to decrease some time after infection and reach the insignificant titre of latent infection in the less severe cases after about a year. In more severe cases they last longer, but, as has been shown by Sabin, also in severe cases which later become fatal, only very low titres may be demonstrable after several years. It is not possible to distinguish by serological means alone whether such titres are the remnants of a congenital disease or a sub-clinical acquired infection This means that it cannot be determined on the basis of such serological reaction whether the congenital cerebral defect is of toxoplasmic origin, or whether the sick child has undergone a post-natal sub-clinical infection fortuitously. The solution of this problem is particularly difficult when the defect syndrome does not include the fairly typical chorioretinitis, and when there are no intracerebral calcifications. There are doubtless severe cases where these symptoms are not found, and in less severe cases they may be found quite infrequently. The conditions following relatively slight pre-natal toxoplasmic encephalitis are therefore simultaneously oligosymptomatic, are recognizable only a long time after birth, and could not be expected to show high antibody titres. Thus it will occur that an uncharacteristic congenital cerebral defect as the result of a pre-natal toxoplasmic encephalitis, which in the toxoplasmosis tests may have the same titres as some normal children, may have to be diagnosed.

This problem can be solved in a satisfactory and certain manner. Comparison should be made between the frequency of positive reactions in a large number of normal children and children with post-natally inexplicable damage

to the brain. It is of great importance that the material should include children with low antibody titres, as otherwise a series of errors will occur. Of course, in view of the greater incidence in older age-groups, comparison should be made only of persons of the same ages. The year-groups should be as small as possible. Similar average ages in the groups compared do not form a suitable basis for comparison.

TABLE I

Comparison of the frequency of positive reaction (Sabin Feldman test) in children with congenital cerebral defects and normal children of the same ages

Age	With brain disease				Normal			% Diff.	σ Diff
	—	+	%+	σ	%+	(%+)	σ		
< 1 yr.	58	12	17	4.5	0.0	(0 0)	–	17	–
2– 5 yrs.	141	29	17	2 9	1.7	(1.1)	1.2	15	3.1
6–10 yrs.	62	25	29	4.9	9 0	(7.4)	3.5	20	6 0
11–14 yrs.	27	10	27	7.3	14 8	(13.9)	6 8	12	10.0
Total .	288	76	20.8	2.1	4 0		1.1	17	2 4

In order to ensure greater reliability of the results, the series of normal children with the higher incidence was chosen for the calculation reported in Table 1 (figures for both series in brackets) Thus the statistical significance is actually greater because of this division of the control material into two halves.

We have carried out such a comparison between 364 cases of children with congenital cerebral defects without chorioretinis or calcifications and twice 300 normal children of the same age (Thalhammer).

It will be seen from Table I that there were more toxoplasma infections among the children with cerebral defects than among the normal children. The difference in the first three age-groups was equally great and was lower in the fourth group. It will also be seen that the curve of this difference is significantly different from the behaviour or the curve for latent infections and must be caused by congenital infections In the 11 to 14 year age-group, the percentage of children with cerebral defect infected with toxoplasma was smaller, despite the higher incidence in normals, on the one hand probably on account of premature death, and on the other because of the cessation of antibody production. From this statistically highly significant difference in the frequency of infections between groups of children with congenital cerebral defects and normal children, it can be concluded that among cases of uncharacteristic congenital demage to the brain, such as mental retardation and epileptic-like fits, a certain number (in our study 17 per cent.) are due to relatively slight pre-natal toxoplasmic encephalitis.

On the basis of these findings, diagnosis is possible in individual cases by means of calculation of the probability. In cases of congenital cerebral defects with positive reactions, the probability of a merely fortuitous coincidence of a cerebral defect with a post-natal sub-clinical toxoplasma infection is just as great as the incidence of infections in normal children of the same age.

TABLE II

Incidence of human toxoplasma infections in Vienna
(Frequency of positive Sabin-Feldman reactions among normal persons)

Age in years	<1	2-5	6-10	11-14	11-20	21-30	31-40	41-50	51-60
No. tested (both series)	147	270	135	55	139	228	185	154	121
% positive (both series) ...	0.0	1.1	7.4	13.9	29.7	59.1	62.3	68.8	66.9
% positive (higher series) .	0.0	1.7	9.0	14.8					

Under the conditions in Vienna, the diagnosis of oligosymptomatic congenital toxoplasmosis can be made among children in their first year of life with 100 per cent. certainty, in their second to fifth year with 98 per cent. certainty, in the sixth to tenth year with 91 per cent., and in the eleventh to fourteenth year with 85 per cent. certainty. In older persons, this method of diagnosis is excluded, because the incidence of latent infections increases to above 50 per cent., but in regions where this incidence is low, this method can be used in adults also. In our region, cerebral or eye defects among adults cannot be proved to be of toxoplasmic origin

As is known, freshly-acquired toxoplasmosis can only be diagnosed on the basis of isolation of toxoplasma. In the majority of the other cases, the etiology of an acute disease can only be proved by demonstration of a significant rise in titre. This involves the performance of two tests at an interval of one week; that is to say, the introduction of any therapy proposed must be postponed for a week. This can have serious consequences, as nowadays "blind" treatment is carried out not with sulphonamides but with antibiotics. We believe that a method does exist for recognizing or excluding acquired clinical manifest toxoplasmosis quickly, provided that the physician is aware of this possibility.

On the first days after appearance of symptoms, also the very sensitive Sabin-Feldman dye test is still negative. Then, at the end of the first or the beginning of the second week of the disease, the test is first positive with low and then with medium titres which thereafter rise rapidly to high values In cases with benign course, the titres decrease to medium and then low values within a few months but remain positive for many years and even for a life-time Low titres can thus be found in both quite fresh and older

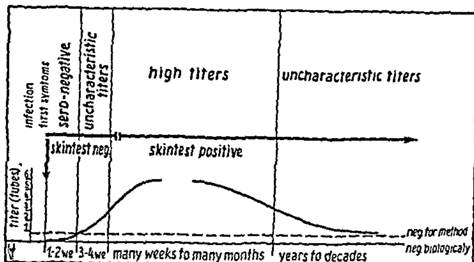


Fig. 1

inactive infections Differentiation between these two stages was previously only possible by means of a titre curve. This would involve the loss of valuable time in introducing therapeutic measures. In our opinion, the combination of the Sabin-Feldman test and Frenkel's skin test prevents this time lapse (Thalhammer, b.c.). It is known from many years' experience with tuberculosis and the few cases of acute toxoplasmosis so far encountered, that the allergy test becomes positive four to five weeks after the infection. Thus a low Sabin-Feldman titre and a positive skin test can only occur at the end of the "general titre curve", several months to years after the infection. Such an infection with toxoplasma cannot be the cause of an acute disseminated illness. However, a low Sabin-Feldman titre and a negative skin test can occur only two to three weeks after infection. Such a combination of reactions makes the existence of an acute toxoplasma infection highly probable, and suggests the advisability of the introduction of therapy. High Sabin-Feldman titre and negative skin test indicate an approximately three to five-week-old infection, and a high titre with positive skin test a more than five weeks old, but still active, infection. The supposition for this method of "discrepancy explanation" is, of course, that the results of the tests are correct. The titre in the laboratory must be at the customary level and the skin test should not seem to be negative because of a weak antigen having been used, or positive because of the antigen being unspecific or contaminated This can be excluded by comparing the titres found known typical cases of infant toxoplasmosis and by correlation between Sabin-Feldman and skin tests. Moreover, it has been observed that the test cannot be used in patients under one year of age, and that this test seem to be negative in very old persons with skin atrophy. We do not

that chorio-allantoic antigen is suitable for use in the skin test, since it cannot be sufficiently concentrated, and because it contains egg albumin. Various workers have observed poorer correlation between the results when using chorio-allantoic antigen than when using mouse ascites antigen in the skin test. We have found the correlation to be at least 97 per cent.. As regards the possible objection that the injection of the antigen evokes a rise in titre, thus diminishing the value of the titre curve, I would state that up to now I have not seen a significant rise in titre (more than one tube) after a skin test, and that the differentiation of the age of the infection is already obvious after the first combined test, so that a possible subsequent rise in titre plays no great role.

TABLE III

Results of test for toxoplasmosis in a 12-year-old boy

Date	Sabin-Feldman	Complement fixation	Skin test	Duration
23/3	1 64	1 64	–	3 weeks
7/4	1 4096	–	neg	5 weeks
28/4	1 16384	> 1 128	pos	8 weeks
10/6	1 16384	–	–	14 weeks
29/12	1 256	–	–	43 weeks

Such acute infections with toxoplasma are seldom available for testing, but I am able to show you a case in which the utility of the method mentioned here seems to be proved (Table III). The child was suffering from a severe bilateral iridicyclitis with slight tumour of the spleen (Pillat, Thalhammer). One year after the commencement of the illness, one eye was enucleated on account of hypotonia. Animal isolation experiments with iris emulsion were positive in the first passage, and toxoplasma-like bodies were demonstrated by histology. The Sabin-Feldman test showed primary insignificant titres, but the complement fixation test was already positive Two weeks later the tests were repeated. The titre had risen considerably, but the skin test made at that time was negative A further three weeks later the skin test was positive, and the titre had risen by one tube and remained at that level for at least two months. Six months later the titre had decreased significantly.

The combination of the Sabin-Feldman and complement fixation tests is not suitable for early diagnosis, since, as is known, low dye test titres are often found together with negative complement fixation reactions in old infections. The statement (Sabin) that the complement fixation test becomes positive at a later stage and negative at an earlier stage than the Sabin-Feldman test (other antibodies being involved) could not be confirmed for

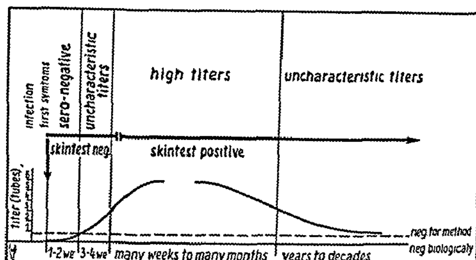


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tions are not too much in a toxoplasmosis complement fixation test, and the 3.8 per cent. doubtful results are found just as frequently in the Sabin-Feldman test as in the complement fixation test, and only in weakly-positive, definitely doubtful, positive sera. Among the 324 clearly useable double tests, there were 10 per cent. discrepancies. These also occurred only in weakly-positive sera and the Sabin-Feldman test was negative just as frequently as the complement fixation test, thus indicating that the discrepancies are not entirely due to the weakness of the complement fixation test. The last column but one on the right of the table is of importance for the use of the complement fixation test. It will be seen that of the sera with dye test titres of 1.4, 62 per cent. were positive in the complement fixation test; of the sera with dye test titres of 1:16, 90 per cent. were positive, and of the sera with dye test titres of 1·64 or above, 100 per cent. were positive in the complement fixation test Thus, using the complement fixation test and dried antigen, active toxoplasma infection could be determined in all cases. For this purpose, the complement fixation test with dried antigen is very suitable. The last column on the right of the table shows the frequency of the different Sabin-Feldman titres among 578 positive normal persons, 44 per cent. of these had titres of 1:4 and 40 per cent titres of 1.16, while higher titres were found in only 15 per cent. The combination of the values in the last two columns shows that, by the use of our antigen, we were able to demonstrate latent infection in only 78 per cent of the cases. Therefore, for determination of the incidence of toxoplasma infection and for elucidation of the etiology of defect conditions from long-expired infections, the complement fixation test cannot be used alone, but in conjunction with the skin test Using the same antigen, this indicates at least 97 per cent. of the infections of more than 5 weeks' duration.

The thermostabile dry antigen, which is comparable to the best thermolabile fluid antigens, was used according to the Kolmer method, in a dilution of 1:1300 This means that each mouse provided about 11 ml antigen diluted for use. The dilution used for the skin test was 1:5000 It is important to use a good complement. It is thus obvious that the activity of the dry antigen is dependent on the quality of the original fluid material

To conclude, it can be stated that provided a good technique and a durable antigen equivalent to ours are used, sero-diagnosis of toxoplasmosis can be carried out in the general laboratory. In certain cases the complement fixation test should be combined with the skin test, and in doubtful cases the Sabin-Feldman test should always be performed.

Finally, a brief word concerning what seems to be an unsolved problem. It is known that particularly in adults numerous cases of focal chorioretinitis are found, the origin of which is unknown, or which are more or less unproved and classified as tuberculous. The ophthalmologists are aware of this problem

the complement fixation test with our antigen. However, it should be stated that, also when using our antigen, it was not possible to obtain complement fixation reactions with all sera which showed low Sabin-Feldman titres.

As regards the third problem of the sero-diagnostic methods in toxoplasmosis in the general laboratory, only the question of the complement fixation test will be mentioned here. In order to enable all laboratories to perform this test, an antigen must be made available which is not only potent and specific, but also thermostabile and thus suitable for transport and storage. The usual fluid antigens do not meet the latter condition; they can only be stored in deep-freezer and thus cannot be easily transported As a result, each laboratory must produce its own antigen.

Westphal has tried to produce a durable fluid ascites antigen He has, in fact, been successful, though the production process is complicated and much antigenic substance was lost, resulting in too weak an antigen. In addition, at least one batch was contaminated with typhus antigen The investigations of Afzelius-Alm and Hahn, Scholta and Kulasiri showed that this antigen was unusable

A strong, thermostabile antigen is still lacking For the last three years we have been carrying out experiments in this direction and believe that a simple solution has been found (Thalhammer, d.) A mouse ascites antigen produced after the method of Steen and Kass was lyophilized This did not alter in activity when stored in vacuum ampoules at 21 to 28° C for at least seven months

The value of a toxoplasmosis complement fixation antigen is expressed by the extent to which the tests are in agreement with Sabin-Feldman tests carried out simultaneously. We have performed such tests with dried antigen on 354 sera (Table IV). It will be seen that we regard sera with titres of 1:4 and 1:16 in the Sabin-Feldman reaction as positive, and consider them to be indicative of previous infection. 4.8 per cent anti-complementary reac-

TABLE IV

Comparison of results of Sabin-Feldman test with dried antigen in 354 sera

S-F	1:2	1:4	1:8	1:16	1:32	1:64	Neg	?	Anti-compl.	Total	%	(*)
1:4	14	5	1	2			13	1		36	62	44 %
1:16	15	26	12	10	2		7	1	6	79	90	40 %
1:64	3	16	18	10	4	3			4	58	100	13 %
1:256				2	2	4			1	9	100	2 %
Neg.	10	1	2				142	2	6	163	91	
?	2						6	1		9		
Total...	44	48	33	24	8	7	168	5	17	354		

* *Titre distribution among 578 positive normal persons*

tions are not too much in a toxoplasmosis complement fixation test, and the 3.8 per cent. doubtful results are found just as frequently in the Sabin-Feldman test as in the complement fixation test, and only in weakly-positive, definitely doubtful, positive sera. Among the 324 clearly useable double tests, there were 10 per cent discrepancies. These also occurred only in weakly-positive sera and the Sabin-Feldman test was negative just as frequently as the complement fixation test, thus indicating that the discrepancies are not entirely due to the weakness of the complement fixation test. The last column but one on the right of the table is of importance for the use of the complement fixation test. It will be seen that of the sera with dye test titres of 1:4, 62 per cent. were positive in the complement fixation test; of the sera with dye test titres of 1.16, 90 per cent. were positive, and of the sera with dye test titres of 1 64 or above, 100 per cent. were positive in the complement fixation test Thus, using the complement fixation test and dried antigen, active toxoplasma infection could be determined in all cases For this purpose, the complement fixation test with dried antigen is very suitable The last column on the right of the table shows the frequency of the different Sabin-Feldman titres among 578 positive normal persons; 44 per cent. of these had titres of 1.4 and 40 per cent titres of 1:16, while higher titres were found in only 15 per cent. The combination of the values in the last two columns shows that, by the use of our antigen, we were able to demonstrate latent infection in only 78 per cent of the cases Therefore, for determination of the incidence of toxoplasma infection and for elucidation of the etiology of defect conditions from long-expired infections, the complement fixation test cannot be used alone, but in conjunction with the skin test Using the same antigen, this indicates at least 97 per cent. of the infections of more than 5 weeks' duration

The thermostabile dry antigen, which is comparable to the best thermolabile fluid antigens, was used according to the Kolmer method, in a dilution of 1.1300 This means that each mouse provided about 11 ml antigen diluted for use. The dilution used for the skin test was 1 5000. It is important to use a good complement. It is thus obvious that the activity of the dry antigen is dependent on the quality of the original fluid material

To conclude, it can be stated that provided a good technique and a durable antigen equivalent to ours are used, sero-diagnosis of toxoplasmosis can be carried out in the general laboratory. In certain cases the complement fixation test should be combined with the skin test, and in doubtful cases the Sabin-Feldman test should always be performed.

Finally, a brief word concerning what seems to be an unsolved problem. It is known that particularly in adults numerous cases of focal chorioretinitis are found, the origin of which is unknown, or which are more or less unproved and classified as tuberculous The ophthalmologists are aware of this problem

and previously presumed that a number of these cases were caused by toxoplasmosis. However, they were not able to confirm this. Histological examination of the chorioidea is only seldom possible, and many of the tests carried out are positive, as they are in many normal persons. It has not been possible to show a statistically significant difference because clinical evaluation of the material was impossible In such circumstances, the basic work of Jacobs et al. was of great value. Enucleation was made of the eye of a man who had been suffering for eight years from definite acquired, severe active chorioretinitis. In animal experiments and by histological examination, a definite case of toxoplasmosis was established, and thus the existence of acquired toxoplasmic chorioretinitis was proved. However, before the operation a titre of 1 64 had been found on several occasions in the Sabin-Feldman test, i. e. a titre which can be found in many normal persons of similar age. Thus it would have been impossible to make a definite diagnosis in this case by serological methods. In contrast to this disease of eight years' duration with only *local* relapses, a case reported by Wising, with fresh chorioretinitis and lymphadenopathy, showed a titre curve characteristic for toxoplasmosis Therefore, it could be assumed that also the case reported by Jacobs could have been diagnosed by serological means in the first stages of the disease. Then this case would show only that such local relapse, caused by the bursting of pseudocysts, giving symptoms in the eye alone or perhaps in the brain, would not be accompanied by a rise in titre. However, in many cases of acute general toxoplasmosis, post mortem examination reveals pseudocysts in many clinically unaffected organs That is to say, in certain circumstances – in cases with a small number of organisms and good local resistance – the primary invasion of the germs can occur without any symptoms or signs becoming apparent. If, however, this takes place in the chorioidea with subsequent bursting of a pseudocyst, the first clinical manifestation of chorioretinitis biologically will be a local relapse accompanied by uncharacteristic antibody titres. It should be mentioned that the frequency of such cases is not known. I do not know whether such a case could be diagnosed. Perhaps it would be possible clinically if the ophthalmologists were enabled to obtain sufficient experience concerning changes in the fundus of proved cases.

SUMMARY

Four difficult problems connected with diagnosis of toxoplasmosis are discussed: (1) The etiological evaluation of uncharacteristic cerebral defects from congenital toxoplasmosis (without chorioretinitis and without calcifications) is possible only by means of statistical comparison of the incidence of infection in normal children and in those with congenital damage to the brain. In individual cases the diagnosis is possible with a probability of 100,

minus the infection rate for normal children of the same ages. (2) Cases of acute acquired toxoplasmosis in the stage of low or medium titres could only be diagnosed by titre curves with much loss of time. These can be discovered within 48 hours, based on the fact that the low and medium dye test titres with a negative skin test reaction only appear within the first two or three weeks after the infection, while such titres indicating previous infection are accompained by a positive skin test The antigen and technique used are important and are discussed. The combination of the dye and complement fixation tests does not serve the same purpose, as positive dye test and negative complement fixation test occur both in fresh and in old infections (3) For sero-diagnosis outside the special laboratory, a potent, specific antigen which remains stable at room temperature, is necessary for use in the complement fixation test. Use of a dried thermostabile antigen made in Vienna showed that of the sera with dye test titres of 1:4, 62 per cent. were positive in the complement fixation test; of the sera with dye test titres of 1.16, 90 per cent. were positive, and of the sera with dye test titres of 1.64 or above, 100 per cent. were positive in the complement fixation test The antigen is thus suitable for diagnosis of all still active cases. For establishment of the incidence of toxoplasma infections and for evaluation of the old defect conditions (low titre), the complement fixation test must be combined with the skin test. (4) Local relapse because of bursting of pseudocysts long after the infection is accompained by medium or low antibody titres but may cause acute clinical symptoms in the eye (and perhaps also in the brain). Serological diagnosis of such cases is not possible because of the high infection rate among normal adults, and therefore their incidence is not known.

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Department of Pathology and Oncology, University of Kansas School of Medicine, Kansas City, Kansas, U.S.A.

REVIEW OF ORGANISMS RESEMBLING TOXOPLASMA

J. K. Frenkel

A number of organisms may resemble *Toxoplasma* morphologically, in that they are of similar small size and simple organization. Biologic criteria, especially serologic and immunologic behavior, host range and host response to infection, furnish the more useful criteria for identification. However, morphologic data are useful and are frequently the only ones available.

The following organisms infecting man have been confused with *Toxoplasma: Histoplasma, Cryptococcus (Torula), Sarcocystis* and the leishmaniform stages of *Trypanosoma* and *Leishmania*. In addition the following organisms present differential diagnostic problems in animals. *Encephalitozoon, Klossiella* and other Coccidia, *Besnoitia* and an organism in field mice of the genus *Microtus*.

Two forms of *Toxoplasma* are distinguished. proliferative forms (Figs 1 and 2) and cyst (or pseudocyst) forms (Figs. 3 and 4) *Proliferative forms* are found during the acute stage of toxoplasmosis. Their morphology can easily be studied in the peritoneal fluid of mice after intra-abdominal inoculation of organisms. They are crescentic in shape and measure about 3×5 micra. One end near the nucleus is rounded, the other is pointed and motile When this peritoneal exudate is spread into a thin film, dried, covered with methanol and stained with Giemsa's stain this morphology is observed. After fixation, especially in tissue cells, *Toxoplasma* frequently appears ovoid or spherical In general organisms are adequately demonstrated by hematoxylin and eosin, although staining with Giemsa's of sections may facilitate their recognition, the cytoplasm is densely stained with methylene blue but only slightly with eosin. Within the cytoplasm a few granules smaller than the nucleus can be found that stain after treatment with periodic acid and leukofuchsin (PAS). These behave like glycogen in that they are digested by diastase. Gustafson, Agar and Cramer (1) have described the ultrastructure of *Toxoplasma* as observed under the electron microscope. Multiplication of organisms occurs intracellularly, by multiple binary fission The "final stage of leukocytic parasitization by toxoplasma" has been called a "terminal colony". The host cell disintegrates and the organisms liberated enter other cells, and go through a similar proliferative cycle in susceptible, non-immune hosts

Cysts of Toxoplasma are found during chronic infection. Characteristically they are present in the brain, eye, myocardium and skeletal muscle and if intact are usually unaccompanied by cellular reaction. They may persist for long periods of time. Cysts arise from proliferative forms in the later stages of acute infection, during sub-acute and possibly during chronic infection. Cysts in the brain and eye are spherical and measure from 30–100 micra in diameter (Fig. 4). Within skeletal and cardiac muscle, cysts are elongate (Fig. 3). Cysts are surrounded by a resilient membrane, less than a micron in thickness, which is thought to be of parasite origin, just like the coccidian oocyst membrane (Fig. 4). The cyst membranes of *Toxoplasma* and of the Coccidia are argyrophilic and PAS-positive (not due to glycogen). The nucleus of the host cell can frequently be seen, indicating that cysts develop intracellularly. Each cyst contains numerous thin crescentic organisms 2×6 micra in diameter. These cyst-forms of *Toxoplasma* each contain a glycogen granule, about the size of the nucleus (1–2 micra). After PAS-staining, the entire cyst assumes a red tinge, and therefore it is easily seen in sections even at low power magnification (Fig. 3).

Cyst rupture gives rise to necrotizing lesions, suggesting hypersensitivity (Fig 3). Cyst rupture in the retina is believed to account for the recurrent attacks of chorioretinitis in man Most, if not all, organisms liberated from cysts are destroyed; however, under the influence of pharmacologic doses of corticoids, immunity may be so depressed that such liberated organisms can enter and proliferate in new cells.

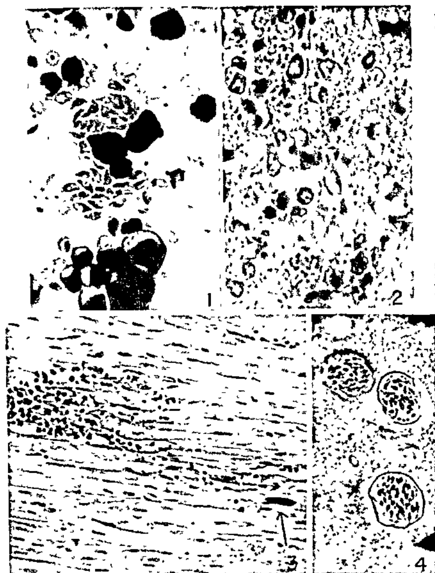
Histoplasma is getting to be more commonly recognized outside of the Americas. This fungal organism when parasitic, generally grows in the yeast form, usually within phagocytic cells of lymph nodes, spleen, liver (Fig. 5), lungs and bone marrow. PAS-staining furnishes a satisfactory means of differentiation, in that the cell membrane of each organism is stained and glycogen granules are absent (Fig. 5). Rarely mycelial forms are found in tissues; however, it is these stages that provide the identifying characteristics when *Histoplasma* is cultured *in vitro* at room temperature.

Figure 1. Toxoplasma, proliferative forms in impression smear from lung of a mouse Air dried Methanol, Giemsa.

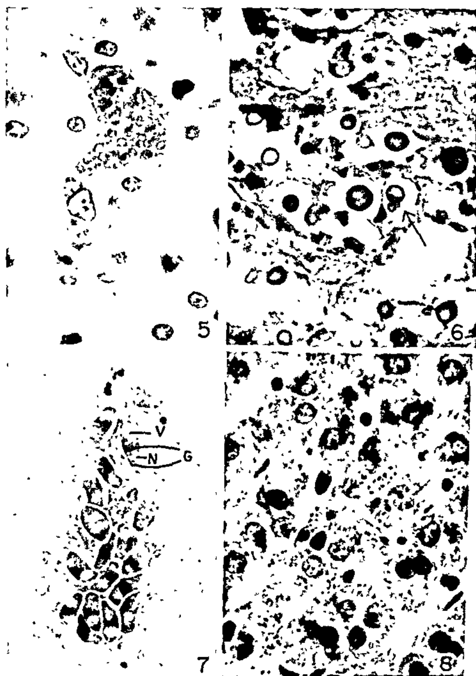
Figure 2. Toxoplasma, proliferative forms in focus of acute encephalitis of hamster. Zenker's fixative. Periodic acid-Schiff, hematoxylin

Figure 3. Toxoplasma cyst (arrow) in myocardium, darkly staining due to its glycogen content, unaccompanied by cellular reaction. The inflammatory focus on the left presumably followed rupture of a cyst several days previously. Periodic acid-Schiff, hematoxylin (section courtesy of Donald B Hackel)

Figure 4. Toxoplasma cysts in brain of mouse with a chronic infection Silver impregnation demonstrates the semi-rigid cyst wall. Wilder's silver impregnation.



Figures 1, 2, 4 magnified 800 × Figure 3 240 ×



Figures 5–8 magnified 850 ×

Cryptococcus (Torula), another fungus, is usually found in the skin, lungs or brain, and a slowly progressive meningo-encephalitis may be produced. *Cryptococcus* shows variability in size and occasional budding (Fig. 6). The cell membranes are PAS-positive, similar to those of *Histoplasma* In addition a mucopolysaccharide capsule is present, which can be demonstrated best by means of mucicarmine (2) In fresh spinal fluid the cryptococci may resemble lymphocytes; however, their capsule can be demonstrated since it stains negatively when the cerebro-spinal fluid is mixed with India ink.

Sarcocystis is a protozoan organism usually found encysted in cardiac and skeletal muscle of such hervivores and omnivores as sheep, cows, ducks, guinea pigs, rabbits, mice and man. The developmental cycle is not known Focal myocarditis and myositis, sometimes widespread, are produced by the breakdown of cysts. The cyst walls are argyrophilic but PAS-negative Some species such as those from cottontail rabbits, show radial spines. "Sporoblastic" cells are often found inside the cyst wall The cysts of some species contain compartments and the central ones may be free of organisms The individual cyst organisms are large and have two rounded ends (Fig 7). Cyst organisms are motile, have vesicular nuclei and contain many glycogen granules with a characteristic perinuclear distribution (Fig 7) Unlike fungi, individual organisms are not surrounded by a PAS-positive wall They react with specific cytoplasm-modifying antibody in a fashion similar to *Toxoplasma* The occurrence of schizogony is doubtful. Morphologic studies of organisms from man, sheep, mouse, rabbit, squirrel and duck did not reveal fungal characteristics. The few accounts of transmission by subinoculation or feeding of cyst organisms are poorly substantiated Such attempted transmissions of mouse and rabbit *Sarcocystis* to laboratory mice and rabbits were unsuccess-

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Figure 5. Histoplasma capsulatum in Kupffer cell of mouse Note the distinct outline of the yeasts by the periodic acid-Schiff method The nuclei are stained by hematoxylin

Figure 6 Cryptococcus neoformans in meninges The individual yeasts are usually larger than *Histoplasma* Budding is indicated by arrow The nuclei are obscured by the densely staining yeast wall The polysaccharide capsule has shrunken and is represented by the clear space This capsule may not be as pronounced as shown here, and can be stained by mucicarmine Periodic acid-Schiff, hematoxylin

Figure 7. Sarcocystis from rabbit. A cyst was dissected from muscle and ruptured artificially to demonstrate the individual organisms. The impression smear was stained with the periodic acid-Schiff method, demonstrating the glycogen granules (G) near one end and surrounding the negatively staining nucleus (N) Vacuoles (V) are demonstrated by cytoplasmic staining according to Giemsa

Figure 8. Leishmania in Kupffer cells of hamster. The nuclei are represented as dots, the kinetoplast is too small to be reproduced, but it is easily identified by direct microscopy under oil immersion Hematoxylin and eosin staining No additional structures are brought out by the periodic acid-Schiff method

ful in the author's hands. *Sarcocystis* from cottontail rabbits and house mice also failed to grow on media ordinarily supporting fungal growth. It appears from these studies that these organisms neither look nor behave like fungi.

The leishmaniform stages of *Trypanosoma cruzi* and *Leishmania* (Fig. 8) can be differentiated by the presence of a kinetoplast (often called blepharoblast or parabasal body), a small rod-like structure in the cytoplasm, densely staining with hematoxylin. *Leishmania* are smaller than *Toxoplasma.*

Encephalitozoon has frequently been confused with *Toxoplasma*, and is regarded by some as a species of *Toxoplasma*. However, it should be conceptually separated from the latter, pending proof of identity. The organisms are found predominantly in the brains of laboratory mice and the brains and kidneys of laboratory rabbits and guinea pigs. *Encephalitozoon* is smaller, ovoid, stains poorly with hematoxylin, but readily with carbol-fuchsin Although organisms may remain in the host for long periods of time, a cyst wall is not apparent *Encephalitozoon* has been encountered during "blind passage" of animal tissues and the single, most helpful sign of its recognition is the appearance of a viscid peritoneal exudate in mice, two to three weeks after inoculation. Perrin (3) has compared it with *Toxoplasma* in detail.

Klossiella is a coccidian protozoan Coccidia multiply by schizogony, giving rise to merozoites. Oocysts containing sporocysts and sporozoites are formed in the sexual cycle. The developmental stages of *Klossiella* are found in the kidneys of mice and guinea pigs Other species of Coccidia are found in the lining of the intestine and biliary tract of many mammals and birds, and their crescentic merozoites have been confused with *Toxoplasma.*

Besnoitia organisms, whether proliferative or cyst forms, resemble *Toxoplasma* markedly. However, large cysts up to 2 mm in diameter are formed, which are enclosed in a heavy cyst wall, lined by large nuclei. *Besnoitia jellisoni* has been described recently in deer mice (Peromyscus) from Idaho, U.S.A. (4). In cows and horses from southern Europe and South Africa, *Besnoitia (Globidium) besnoiti* has been known for almost half a century (5). Similar organisms have been described in chickens as Bangkok disease, and in reindeer from Alaska as *Fibrocystis tarandi*. Although the cysts of *Besnoitia* are distinctive on account of their great size and the presence of a heavy cyst wall, lined by nuclei, the proliferative organisms from the acute infection and the lesions produced are almost indistinguishable from *Toxoplasma*. Infection in man has not been observed, but a variety of laboratory animals such as mice, hamsters, rats, guinea pigs, chick embryos can be experimentally infected.

Organisms from Microtus. An organism was observed in the brains of field mice *(Microtus modestus)* from Montana, U.S.A. The distinguishing feature is its cyst, which is lobulated and septate in the brain, whereas that of *Toxoplasma* is spherical and aseptate. This organism does not give rise

to infection when subinoculated into other *Microtus*, white mice, hamsters, or guinea pigs (6). Findley and Middleton (7) observed an organism with similar characteristics in *Microtus agrestis* from England. They believed this to be *Toxoplasma* and attempted to implicate it as causing a fatal epidemic in these field mice. However, no significant encephalitis was present and transmission experiments with organisms from the lobulated cysts appeared inconclusive.

Further illustrations, more detailed discussion of these organisms, and of the pathogenesis of infection produced by these, can be found in another paper (5) published with the *Toxoplasma* symposium of the New York Academy of Sciences.

SUMMARY

The diagnostic features as presented in the laboratory or at post-mortem examination are compared of. *Toxoplasma, Histoplasma, Cryptococcus, Sarcocystis*, leishmaniform stages, *Encephalitozoon, Klossiella, Besnoitia*, and the organism from *Microtus* For attempts at morphologic identification, hematoxylin and eosin, periodic acid Schiff's-leukofuchsin, carbol-fuchsin, mucicarmin stains, and Wilder's or Laidlaw's silver impregnation are most useful. The better criteria can be obtained from the cyst stages of organisms, if such are formed. Although morphologic data are useful, definitive identifications should be based on biologic criteria pathogenesis, immunologic and serologic comparison

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The Toxoplasmosis Department, The State Serum Institute,
Copenhagen, Denmark

LABORATORY METHODS FOR THE DIAGNOSIS OF CONGENITAL TOXOPLASMOSIS[1]

KNUD AAGAARD

The diagnosis of congenital toxoplasmosis demands laboratory support, which is usually obtained in two ways: –

I. The serum antibody titers of mother and child are estimated by the Sabin-Feldman test.

II. An attempt is made to isolate the parasite by inoculation of material from the child into mice.

The Sabin-Feldman Test

Introduction.

Infection by Toxoplasma gondii generally gives the serum of the host ability to kill the parasite in vitro. The parasite killed in this way differs in appearance from the living, and this difference is utilized in the Sabin-Feldman test for measurement of the antibody.

The specific toxoplasma-modifying antibody is thermostable, but demands the presence of a complement-like accessory factor to exercise its effect. This thermolabile factor presents itself quite independently of Toxoplasma gondii e. g. in normal human serum, which is used as source of the accessory factor in the test.

Toxoplasma gondii multiplies in living cells, but here it is not affected by the antibody. That is the reason why the antigen of the test must be rich in extracellular parasites, which, of course, must be living and not connected with disturbing amounts of antibody. These conditions can be fulfilled by the exudate from the peritoneal cavities of mice which have been inoculated with a virulent strain of Toxoplasma gondii.

An alkaline solution of methylene blue is used towards the end of the test, thus making the difference between living and dead parasites clearly visible. Furthermore, both these forms are fixed, and the risk of infection is removed from the mixture.

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Sabin and Feldman made these fundamental observations concerning the toxoplasma-modifying antibody and worked out the first method for its estimation. They called it the dye test, but as the dye only serves a practical purpose, the test is now commonly called after its originators.

Using the original method, the whole test is occasionally a failure, because no appreciable difference can be obtained between the toxoplasma-modifying effect of a positive and a negative serum. In the modified version described below this difficulty is eliminated.

Principles.

The aim of the Sabin-Feldman test is to compare the toxoplasma-modifying effect of different sera. The basis of this comparison is the relationship between the logarithm of the serum dilution and the percentage of modified or killed extracellular toxoplasma.

If the test is successful the graphic picture of this relationship is shaped in the form of an S just like a characteristic dose-mortality curve, indicating that the serum dilution alone decides the percentage of killed or modified toxoplasma in the test tubes.

The serum dilution which modifies 50 % of extracellular toxoplasma (LD 50) is taken as the endpoint of titration Once the laboratory has adopted a definite serum as standard for comparison, it is possible to reproduce relative titer values.

Fortunately the sensitivity of the test can be adjusted in every case so that a fixed endpoint is obtained with the standard serum The relative titer value of the test serum is then identical with its endpoint of titration

Material and Methods

The antigen is obtained from the peritoneal cavities of mice, each of which had been inoculated five days previously with 20,000 extracellular toxoplasma of the RH strain All mice used to supply antigen for the test should weigh no more than fourteen grammes (3 weeks old) at the time of inoculation. Exudate thus obtained is also used for inoculation, but only after two days have elapsed. Consequently the test can be made each time on the same day of the week.

The part of the exudate destined for inoculation is stored in at least three independent containers to ensure one bacteria-free sample. 0.5 ml exudate taken from individual mice with a separate pipette is mixed with 1.0 ml. 0.9% saline and tested for bacterial contamination by culture.

The samples are stored at 4° C and are allowed to clot. The clear fluid round the clot is collected from the non-contaminated samples on the follow-

The Toxoplasmosis Department, The State Serum Institute,
Copenhagen, Denmark

LABORATORY METHODS FOR THE DIAGNOSIS OF CONGENITAL TOXOPLASMOSIS[1]

KNUD AAGAARD

The diagnosis of congenital toxoplasmosis demands laboratory support, which is usually obtained in two ways. –

I. The serum antibody titers of mother and child are estimated by the Sabin-Feldman test.

II. An attempt is made to isolate the parasite by inoculation of material from the child into mice.

The Sabin-Feldman Test

Introduction.

Infection by Toxoplasma gondii generally gives the serum of the host ability to kill the parasite in vitro. The parasite killed in this way differs in appearance from the living, and this difference is utilized in the Sabin-Feldman test for measurement of the antibody.

The specific toxoplasma-modifying antibody is thermostable, but demands the presence of a complement-like accessory factor to exercise its effect. This thermolabile factor presents itself quite independently of Toxoplasma gondii e. g. in normal human serum, which is used as source of the accessory factor in the test.

Toxoplasma gondii multiplies in living cells, but here it is not affected by the antibody. That is the reason why the antigen of the test must be rich in extracellular parasites, which, of course, must be living and not connected with disturbing amounts of antibody. These conditions can be fulfilled by the exudate from the peritoneal cavities of mice which have been inoculated with a virulent strain of Toxoplasma gondii.

An alkaline solution of methylene blue is used towards the end of the test, thus making the difference between living and dead parasites clearly visible. Furthermore, both these forms are fixed, and the risk of infection is removed from the mixture.

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Incubated Mixtures

— A: 0 050 ml. negative specimen + 0.15 ml. mixture A
+ A: 0 050 ml. positive specimen + 0 15 ml mixture A
— B. 0 050 ml. negative specimen + 0.15 ml mixture B
+ B. 0 050 ml. positive specimen + 0 15 ml. mixture B
— C: 0 050 ml. negative specimen + 0 15 ml. mixture C
+ C. 0 050 ml positive specimen + 0.15 ml mixture C

The six tubes with these mixtures are incubated for one hour in a 37° C water bath. A fresh 0.2% solution of methylene blue in a buffer solution with pH 11 is then added to the tubes with 0.10 ml of the solution in each. One drop from each tube is put on a slide and covered with a cover slip The percentage of modified toxoplasms is estimated from a count of 100 extracellular parasites in each sample.

Percentage of Modified Toxoplasms in a Preliminary Test

— A 8	+ A 71
— B· 20	+ B 94
— C. 33	+ C 96

The final test is now made with one part of the antigen and two parts of the accessory factor serum diluted according to the results in the preliminary test. In the given example, this mixture consisted of 8 parts of antigen, 11 parts of accessory factor serum, and 5 parts of 0 9% saline.

Each tube of the final test contains 0 050 ml. serum dilution and 0.15 ml. mixture (antigen + accessory factor serum + saline). For the rest of the test, the tubes are treated just as in the preliminary test. The sera are usually tested in fivefold dilutions, though the standard serum is tested in twofold dilutions. *One series dilutions of the standard is placed at the beginning and* one at the end of the test rack Dilution here means the final dilution in the incubated tubes.

Having added the dye solution to all tubes, samples from each are put under cover slip and placed in a Petri dish with moistened filter paper to prevent evaporation. If the dishes are stored at 4° C, the reading can be postponed for at least three days.

Isolation of Toxoplasma Gondii Avirulent for Mice

The suspected material is inoculated into the peritoneal cavities of mice. The mice should weigh about twenty grammes and belong to an inbred strain frequently controlled for spontaneous infection with Toxoplasma gondii.

A specimen of serum from each mouse is tested for antibody in the Sabin-Feldman test five weeks after the inoculation If the titer of the toxoplasma-

ing day. This fluid contains a small number of toxoplasms, which are nearly all extracellular. The number per ml. is estimated by counting about 400 organisms of the undiluted fluid in a hemocytometer.

Immediately before inoculation the next day, a dilution is made with 0.9% saline, adjusting the number of extracellular parasites to 100,000 per ml.. 0.20 ml of the diluted fluid is inoculated into the peritoneal cavity of each mouse.

The part of the exudate to be used as antigen is mixed thoroughly in a single container, which is stored at 4° C when not in use. The number of extracellular organisms is estimated roughly by counting about 400 parasites of the exudate, which is diluted in the ratio 1:5.

If necessary, the exudate is diluted with 0.9% saline so that the content of extracellular toxoplasma does not exceed 20 million per ml.. No anticoagulant is used in mixing. The homogeneous part of the mixture forms the antigen for the test.

The accessory factor serum of the test is a human serum which contains a sufficient amount of the accessory factor and no traceable toxoplasma-modifying antibody. The serum may be stored at minus 30° C for at least 3 months, and a mixture of different sera can be used if desired.

The original method did not take into account that a rather high concentration of the accessory factor is present in some human sera Such a serum would have been unusable, because more than 10% of the extracellular toxoplasma would have been modified in the tube with a negative control serum.

A serum can be used as source of accessory factor in the test if it is possible to make a mixture of heated (56° C for 30 minutes) and unheated serum that works well; in other words, if the characteristic relationship between the logarithm of the serum dilution and the percentage of modified toxoplasma is obtained with the standard serum.

A preliminary test is made with the antigen and the accessory factor serum to adjust the sensitivity of the system so that the fixed endpoint will be obtained with the standard serum. Three different mixtures of antigen and accessory factor serum are added to both a positive and a negative specimen. If the fixed endpoint of the standard is e.g. 1:4000, then the dilution 1:2000 is taken as positive specimen. 0.9% saline is used as negative specimen.

Preliminary Test

Mixtures of Accessory Factor Serum and Antigen

A. 0 30 ml accessory factor serum + 0 30 ml 0 9% saline + 0.30 ml. antigen
B. 0.45 ml. accessory factor serum + 0.15 ml 0 9% saline + 0 30 ml. antigen
C. 0 60 ml accessory factor serum + 0 ml. 0 9% saline + 0 30 ml. antigen

Department of Serology and Bacteriology, University of Helsingfors, Finland

SOME REMARKS ON THE MECHANISM OF THE DYE TEST

PAUL GRÖNROOS

In September 1948, Sabin and Feldman (9) reported a new staining phenomenon with toxoplasma, later developed into the so-called dye test. In the presence of toxoplasma antibodies and some accessory factors at 37° C, the toxoplasms undergo a change as a result of which they do not stain subsequently with methylene blue. *Sabin and Feldman* (9) showed that the accessory factor was thermolabile and certain complement factors were necessary for the reaction. They assumed that the non-stainability was due to the blockage of certain chromoreceptors.

Roth (8) showed that the complement factors $C'_2 + C'_3 + C'_4$ were necessary to obtain an accessory factor effect.

In an attempt to show that the dye test was non-specific, especially with low serum dilutions, I investigated the toxoplasma hostile factor reported by *Jettmar* (6) This factor can be found in the native serum and its effect on the toxoplasma is the same as in the dye test, *i. e*, the toxoplasms do not stain subsequently with methylene blue This phenomenon can also be observed directly under a phase contrast microscope If sera are inactivated at + 56° C for 5 minutes, the hostile factor disappears If the sera with this factor are investigated in routine dye tests, they are positive or negative without evident correlation with the occurrence of the hostile factor In my study, if less than 50 per cent of the toxoplasms counted were unstained in the original serum dilution $^1/_4$ (final $^1/_8$) in the dye test, the serum dilution involved was considered negative. However, since *Jettmar* used almost undiluted serum, there was a possibility that specific antibodies to low titre were of certain importance for the reaction. The following may throw some light on the problem: A specially chosen serum, which was positive in the Jettmar's test ($^1/_{32}$) (A), gave when inactivated (30 mins at + 56° C) a completely negative Jettmar's test (B), but was positive to $^1/_{256}$ (final dilution) in the dye test (C). Properdin was removed from the native serum by treating it with zymosan at + 17° C for 75 minutes and in addition with zymosan at + 37° C for 15 minutes. With this treatment properdin is removed from the serum as shown by *Pillemer* (7) (D). The serum from D gave the same titre in the dye test when tested without inactivation (E). A drop of con-

modifying antibody is less than 1:10, it is considered proved that no living toxoplasma was present in the inoculated material.

If antibody is found, the titer will nearly always be 1:250 or higher. The brain of the mouse is then suspended in 1 ml. 0.9 % saline and the suspension examined under low magnification for toxoplasma cysts.

This procedure permits the demonstration of one living toxoplasma, even if the strain is avirulent for mice.

Interpretation of Results

A positive Sabin-Feldman test in both mother and child supports the diagnosis of congenital toxoplasmosis, provided the antibody of the child persists beyond the age of four months, thus excluding a passive transference.

Usually titer values of 1.1000–10.000 are found in both mother and child, but lower titers have also been seen.

The isolation of Toxoplasma gondii is of special importance if congenital toxoplasmosis is suspected in a newborn infant and a high titer of the toxoplasma-modifying antibody is found. Toxoplasma gondii has been isolated with the described procedures in such cases, using spinal fluid, blood and even urine as material for inoculation.

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activation of properdin, C'_2 or (C'_1). But even in this case the thermostable factor is necessary. Thus this test series does not suggest that the dye test should be non-specific, which is contrary to what I previously presumed in the same connection, because the thermostable factor seems to be a toxoplasma antibody (3).

Here the question arises as to whether this thermostable factor is always specific. It has not been proved so far, and it is difficult to prove. It is not impossible that certain non-specific reactions could occur. In other reactions investigated so far, the properdin system is of non-specific nature. After Pillemer kindly placed pure properdin at my disposal, it was at last possible to determine the role of properdin in the dye test, as I already have reported (5), and which has subsequently been confirmed by *Feldman* (1). This can be seen in the following table

TABLE II

Titre in dye test (modified)

	Titre in dye test (modified)						
	1/2	1/4	1/16	1/64	1/256	1/1024	1/4096
T+I-serum+P (75 u./ml)	very few Tox			90	86	58	32
T+Buf+P (75 u./ml)	20	15					
T+Buf+Buf	15	15					
T+I-serum+P (30 u./ml) .. .	few Tox		86	96	72	56	16
T+I-serum+P (4,8 u./ml)	96	90	86	82	78	58	36
T+I-serum+P (1,2 u./ml)	86	80	80	72	60	42	
T+I-serum+P (0,6 u./ml)	46	22	20				

T = Toxoplasma suspension in serum lacking properdin (RP), but containing complement.

Buf = Veronal buffer containing Mg++ and Ca++ (pH 7,4)

I-serum = Toxoplasma immune serum Titre in ordinary dye test 1/4096

P = Properdin

The figures give the percentage of "unstained" toxoplasms.

As can be seen, the presence of the properdin system is necessary for the effect of a specific toxoplasma antibody on toxoplasma As far as is known, this is the first time that the role of properdin in a specific antibody-antigen reaction is shown.

As the effect of the properdin system in other reactions is known, it is likely that the effect is directed towards polysaccharides on the surface of the toxoplasma because of which the cytoplasma components stainable with methylene blue escape from the toxoplasma and for this reason, the toxoplasma fails to stain with this substance. The picture seen under a phase contrast microscope also suggested a loss of substance (4).

The quality of the toxoplasma itself also plays an important part in the

centrated properdin (75 u/ml)[1] to one ml of the native serum gave a three tubes higher titre in Jettmar's test (F). A drop of properdin to saline gave a completely negative test (G). A drop of properdin to the accessory factor in the dye test, and performance of the dye test with inactivated serum, did not give higher titre in the dye test (H).

TABLE I

Serum final dilution

	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/102
A	96	82	80	74	58	26	10			
B	32	10	8							
C	96	92	88	86	80	72	58	56	32	
D	30	8	6							
E	98	92	94	86	82	76	60	58	24	
F	96	94	92	86	88	74	70	56	36	12
G	6	4								
H	98	96	90	84	84	76	72	58	34	16

The figures give the percentage of "unstained" toxoplasms
Concerning A, B, C .. see text above.

It would seem that in this case the limiting factor in the Jettmar's test was properdin. After pure properdin was introduced into the test, the limiting factor was the thermostable factor in the serum. This factor seems to be absolutely necessary for the test. With a completely negative serum in the dye test and in the Jettmar's test, it was possible to restore part of the hostile effect to an inactivated Jettmar positive serum It is difficult to prove that the thermostable factor in Jetmar's test is a specific toxoplasma antibody. However, in all the positive sera in the dye test, it was possible to show antibodies in the dye test, except, of course, in undiluted sera which are impossible to measure in the dye test. There was no evident correlation in the titres of the two tests. This is, of course, to be expected, as it seems that in different sera there are different factors which are titrated with the Jettmar's test. As has been shown in other experiments, the limiting factor is very often C'_2.

As I have shown previously, the accessory factor acting in the dye test is the same as properdin + C'_2 + C'_3 + C'_4 and Mg^{++}, $(+ C'_1)^2$ (3). Thus it seems likely that Jettmar's test is a dye test in which the patient possesses the accessory factors, and in which the thermolability depends on the in-

1 [illegible] was made available by *Louis Pillemer*.
2 [illegible]; that C'_1 is also needed for the [illegible] expected in the light of *Wardlaw & Pillemer's* work on the bactericidal activity of the properdin system (11)

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dye test. It is sometimes impossible to obtain sufficiently few positive toxoplasms in the stainability control with an accessory factor serum used earlier or even later. *Schmidtke* (10) reported that she noticed seasonal variations in the usefulness of the accessory factor and test sera for the dye test. I have not observed this, but when using two strains of toxoplasma beside each other for the dye test, there will be times when only one strain is useful for the dye test. Sometimes it takes many passages before the strain is useful again. It is curious that both strains have not been unusable for the test at the same time. The phenomenon is without evident correlation to any external explanation.

It appeared that mechanical agitation of toxoplasma suspension in performing the dye test yielded higher titre for the immune sera. A similar phenomenon was recently reported by *Goldman* (2), who by suspending washed toxoplasma in serum-saline obtained a positive dye test with earlier negative sera His interpretation of this was that these sera have specific antibodies with very low titre, which can be measured with these so-called sensitized toxoplasms only.

The mechanism of the dye test is still not clear. Standardization of routine dye tests seems to be more difficult than expected. It would be of importance to obtain standard sera available in every routine test laboratory.

SUMMARY

A study carried out to ascertain whether the toxoplasma hostile factor reported by *Jettmar* could give non-specific dye test reaction showed that this was probably not the case On the contrary, it was concluded that the phenomenon described by Jettmar for the native serum is to be regarded as a dye test in which the serum itself possesses the accessory factor and the thermostable factor which seems to be a toxoplasma antibody. It could be shown that the thermolability of the reaction was due to the inactivation of properdin and C'_2. It was again demonstrated that even a high percentage of properdin in the test system could not give a positive dye test in the absence of the toxoplasma immune serum.

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DISCUSSION

STUDIES ON THE ACCESSORY FACTOR

H A. Feldman: Our studies indicate that "activator" is composed of properdin, all *four* components of hemolytic complement and Mg^{++} We have not observed "activator" activity in the absence of C'.

The problem which has occupied a good deal of our attention is that of the anti-toxoplasmic activity of fresh "normal" sera obtained from bird and animal species other than man and mice. This was well described in the original paper in which the dye test was first reported and must be borne in mind when testing sera I am certain that many of the difficulties which have been reported with the dye test are due to the fact that fresh, non-heated sera have been used for the experiments Most animal sera have such activity in a titer of about 1.16. We can explain the lack of this non-specific activity in the sera of mice because they are deficient in hemolytic complement and, therefore, do not have activator We cannot explain the fact that human serum has "activator" but, in the absence of antibody, fails to have any action against toxoplasma We have been able to demonstrate recently that in some animal sera, at least, one can dilute the fresh serum to the point where it no longer has any non specific action against toxoplasma but can function as an "activator" when antibody is supplied from another source. The non-specific activity of animal sera can be readily destroyed by heat or by addition of a substance like zymosan which removes some component such as properdin, a portion of hemolytic complement or even Mg^{++} Further studies are in progress to attempt to explain this intriguing property of fresh non-human sera

THE SPECIFICITY OF THE DYE TEST

Leon Jacobs: We have tested 10 rats experimentally infected with *Plasmodium berghei*, another 10 rats infected experimentally with *Trypanosoma cruzi*, 8 chickens experimentally infected with *Eimeria tenella*, 94 squirrels naturally infected with *Hepatozoon* sp., and 3 monkeys naturally infected with *Sarcocystis* sp. None of the experimentally infected animals developed any antibodies against *Toxoplasma*. There was also no relation between the presence of the natural infections with *Sarcocystis* or *Hepatozoon* and the presence of *Toxoplasma* antibodies. We have also found no correlation between the presence of *Trichomonas* in humans and antibodies for *Toxoplasma*.

Eighteen guinea pigs were vaccinated with antigens prepared from *Trichomonas vaginalis* and *T. tenax*. They received from 4 to 7 inoculations. None developed *Toxoplasma* antibodies demonstrable by the dye test. Tests were performed 2 weeks and longer after the last injection.



Fig 1



Fig 2

Dr O Thalhammer Normal toxoplasma and those from negative and positive Sabin-Feldman reactions were examined in the form of sections under the electron microscope (in cooperation with *Braunsteiner* and *Pakesch*) The appearance of normal toxoplasma is, in the main, in agreement with the report published by *Gustafson* The parasites from the negative dye test (Fig 1) show in addition to the methylene blue particules, only slight oedema of the cell, causing separation of the cell membrane from the protoplasm However, the organelles remain unchanged Toxoplasma from the positive dye test (Fig 2) are very much altered The double-contoured cell membrane remains the same, but the protoplasm becomes a homogeneous mass The double contoured membrane of the nucleus remains unchanged also, so that dissolution of the nucleus first takes place when the protoplasm is homogeneous As the dissolution of the protoplasm first takes place in the pointed end of the parasite, and since the toxonemes disappear first, it can

THE SPECIFICITY OF THE DYE TEST

Leon Jacobs. We have tested 10 rats experimentally infected with *Plasmodium berghei*, another 10 rats infected experimentally with *Trypanosoma cruzi*, 8 chickens experimentally infected with *Eimeria tenella*, 94 squirrels naturally infected with *Hepatozoon* sp, and 3 monkeys naturally infected with *Sarcocystis* sp None of the experimentally infected animals developed any antibodies against *Toxoplasma* There was also no relation between the presence of the natural infections with *Sarcocystis* or *Hepatozoon* and the presence of *Toxoplasma* antibodies We have also found no correlation between the presence of *Trichomonas* in humans and antibodies for *Toxoplasma*

Eighteen guinea pigs were vaccinated with antigens prepared from *Trichomonas vaginalis* and *T. tenax* They received from 4 to 7 inoculations None developed *Toxoplasma* antibodies demonstrable by the dye test Tests were performed 2 weeks and longer after the last injection.